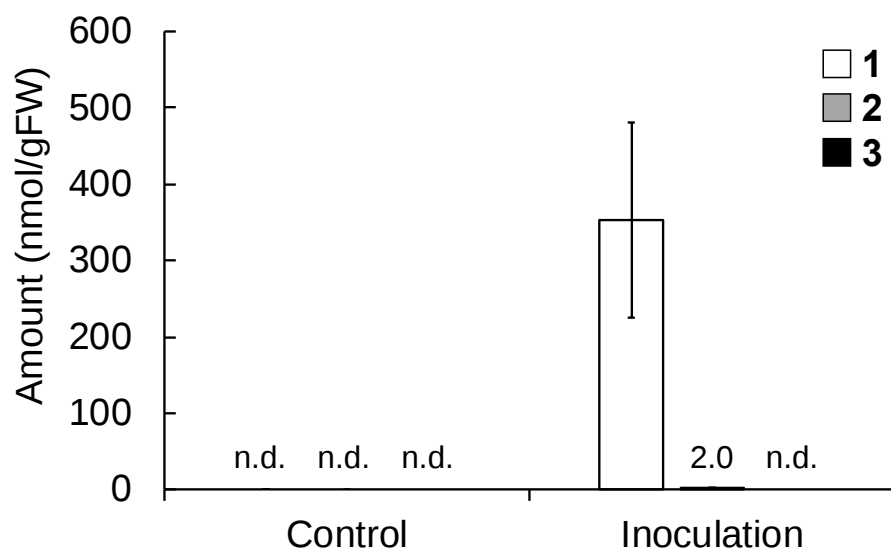


Supplementary materials

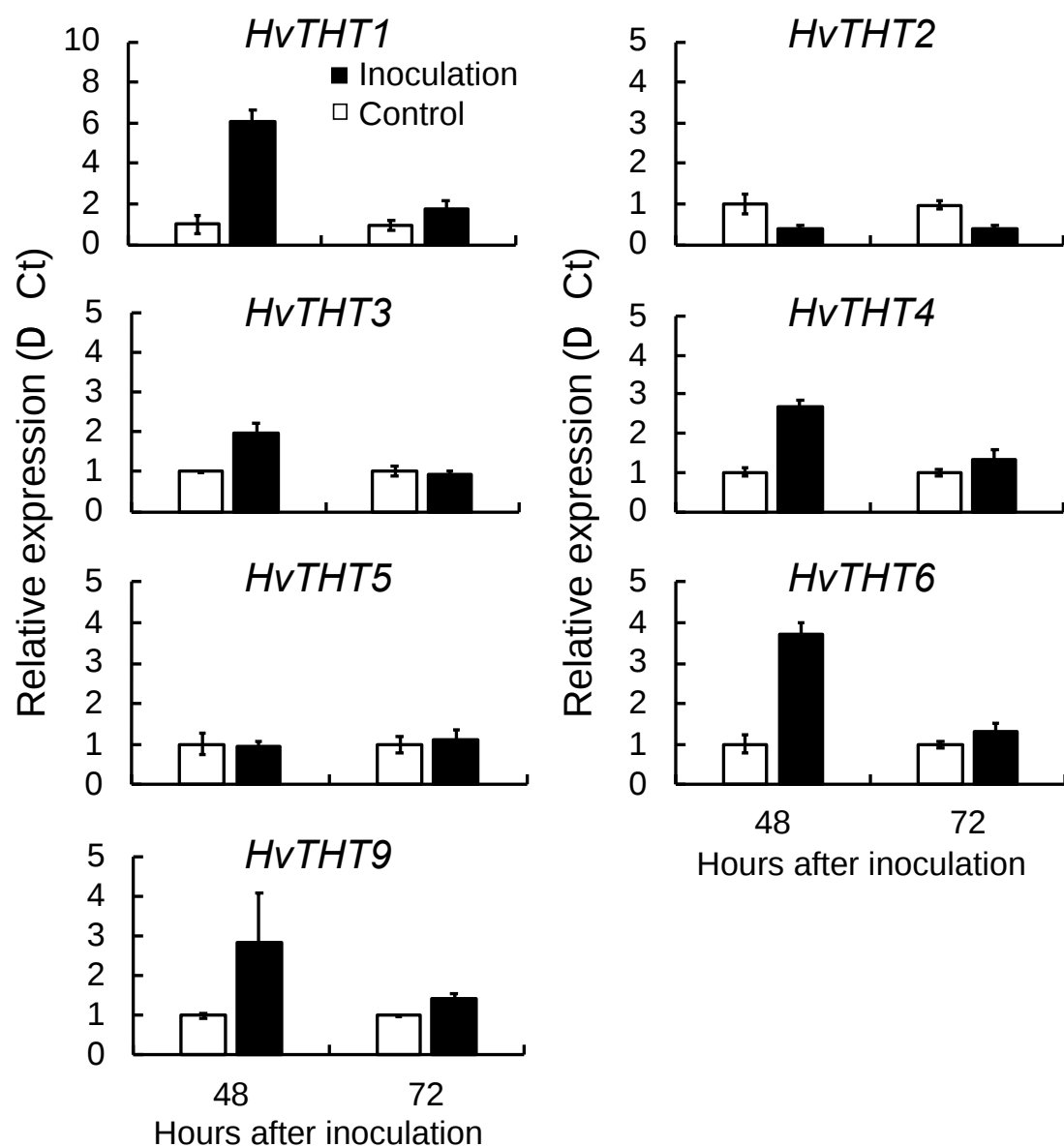
Biosynthesis of Phenylamide Phytoalexins in Pathogen-Infected Barley

Naoki Ube, Yukinori Yabuta, Takuji Tohnooka, Kotomi Ueno, Shin Taketa, Atsushi Ishihara*

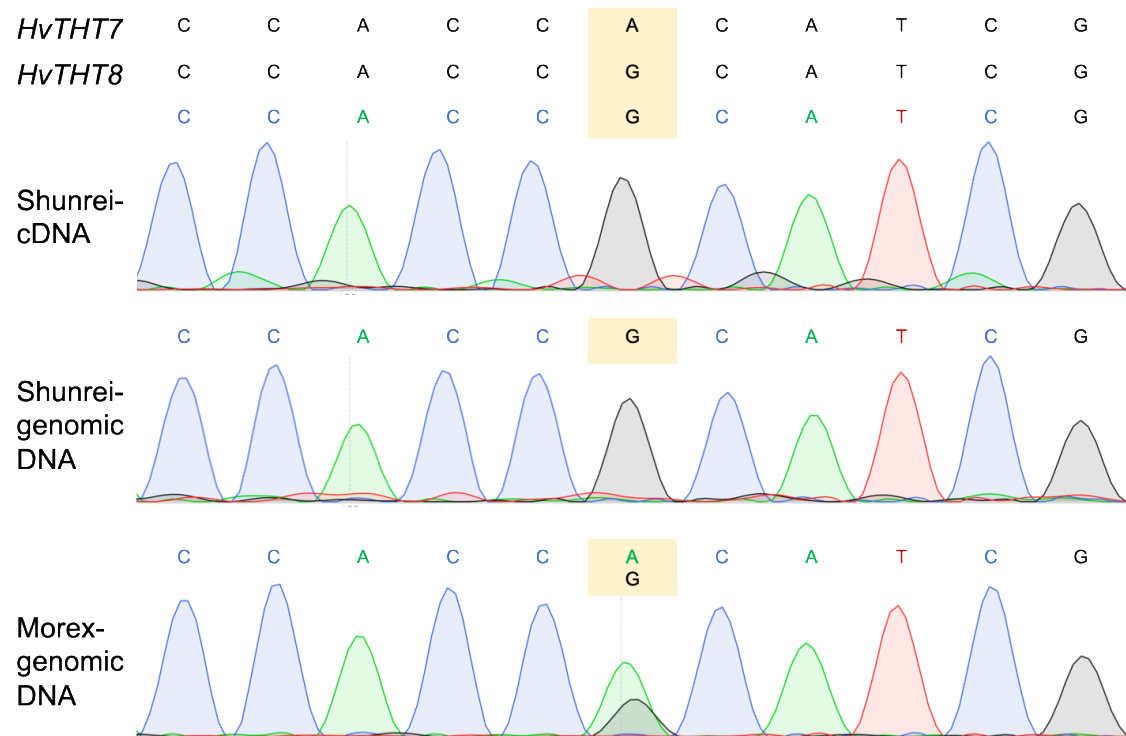
E-mail address: aishihara@tottori-u.ac.jp (A. Ishihara).



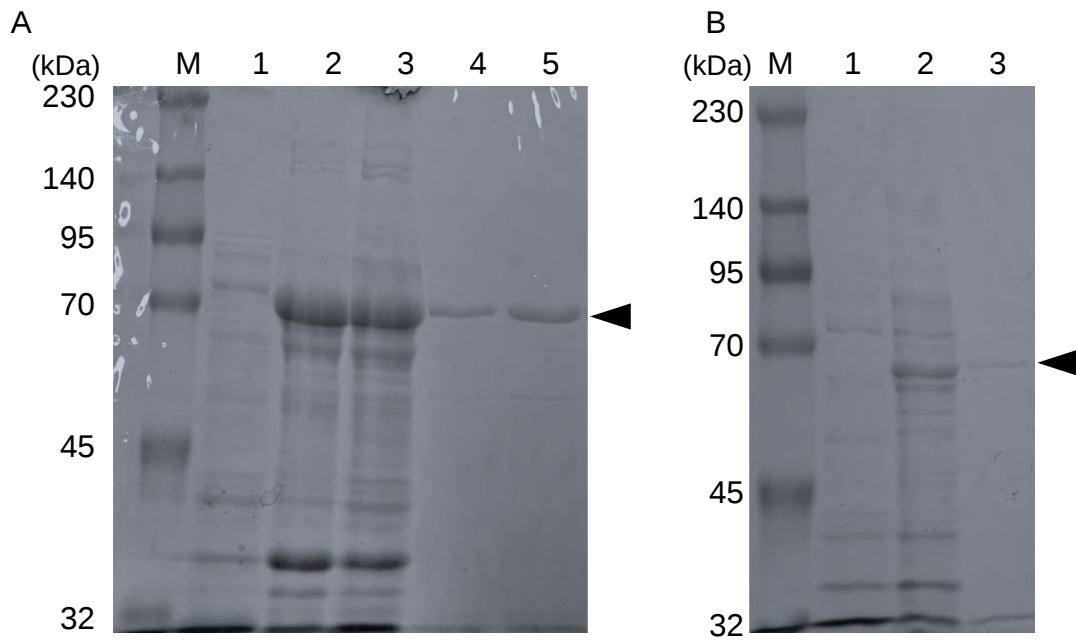
Supplementary Figure S1. Accumulation of compounds **1–3** in *Fusarium culmorum*-infected wheat roots at 72 h after inoculation. Values and error bars represent mean $\pm$ SD ($n = 3$). n.d., not detected.



Supplementary Figure S2. Effects of *Fusarium culmorum* infection on the expression of *HvTHT* genes in barley roots. Total RNA were extracted 48 h and 72 h after inoculation. expression levels were normalized using the ADP-ribosylation factor-like protein (*ADP*) gene as an inner control and are expressed as relative values compared to those of control roots and leaves. Values and error bars represent mean $\pm$ SD ($n = 3$).



Supplementary Figure S3. Sequence analyses of *HvTHT7/8* fragments amplified from cDNA of *F. culmorum*-infected barley ‘Shunrei’ roots and from the genomic DNA of ‘Shunrei’ and ‘Morex’. The green, red, black, and blue lines indicated A, T, G, and C, respectively. The letters above the waves indicated nucleotides around the single nucleotide substitution site of *HvTHT7/8* (490–500 bp from the start codon).

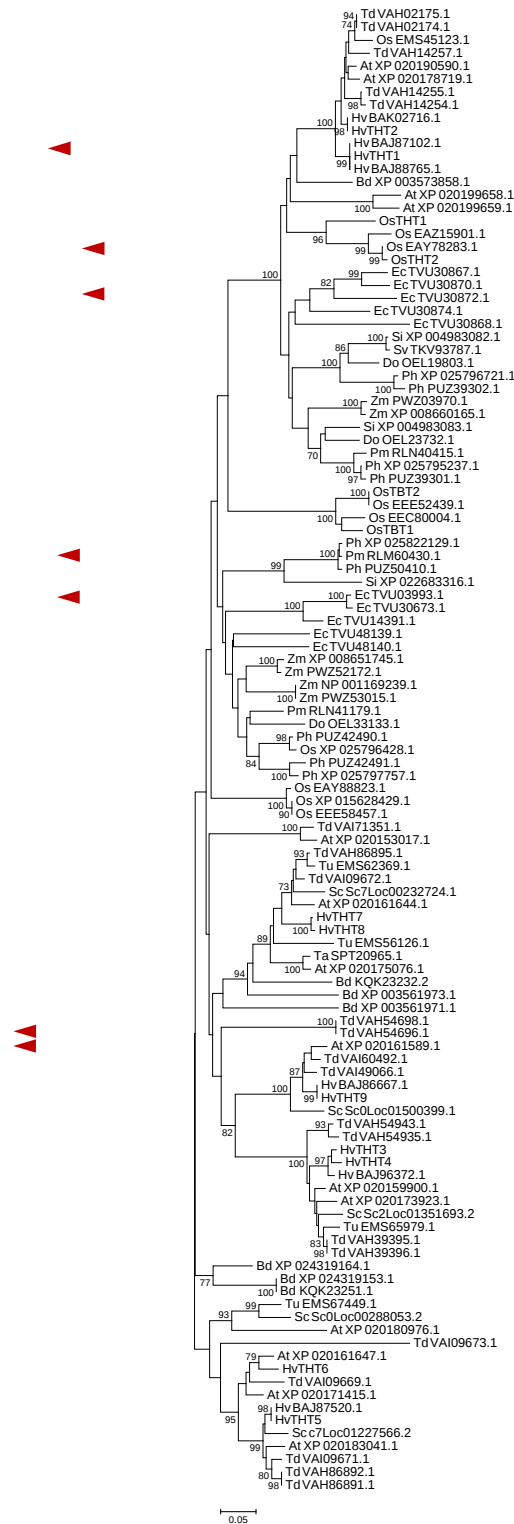


Supplementary Figure S4. SDS-PAGE analysis of recombinant HvTHT2, HvTHT7 and HvTHT8. Proteins from each purification step were separated by SDS-PAGE on an 8.5% gel and stained with Coomassie Brilliant Blue R-250. (A) Lane M, Molecular marker; Lane 1, crude *E. coli* extract; Lane 2, crude extract of *HvTHT7*-expressing *E. coli*; Lane 3, crude extract of *HvTHT8*-expressing *E. coli*; Lane 4, *HvTHT7* purified by metal-affinity chromatography; Lane 5, *HvTHT8* purified by metal-affinity chromatography. (B) Lane M, Molecular marker; Lane 1, crude *E. coli* extract; Lane 2, crude extract of *HvTHT2*-expressing *E. coli*; Lane 3, *HvTHT2* purified by metal-affinity chromatography. Arrowheads indicate HvTHT proteins.

HvTHT2	1	-----MAVMVEITQSMLEPSKESAR-GGGKKVPLIVFDRASTDGYTPAVFAWAP	50
HvTHT7	1	MEVNHAEAGRQVAATSRIAMLKPVYAAPHPLAGGKVQLSVFDRAAIDTYVPIVLAYPAP	60
HvTHT8	1	MEVNHAEAGRQVAATSRIAMLKPVYAAPHPLAGGKVQLSVFDRAAIDTYVPIVLAYPAP	60
HvTHT2	51	APTNAALKAGLVAAVARFPHLAGRFAADDHGRKCFHLNDAGVLVEATADADLADALAHD	110
HvTHT7	61	APSNEALKEGLLRATAPYPHLLGRFALDAHGRRLHLNNEGVLVIEADVPADLADVLAAG	120
HvTHT8	61	APSNEALKEGLLRATAPYPHLLGRFALDAHGRRLHLNNEGVLVIEADVPADLADVLAAG	120
HvTHT2	111	-VSAHINELYPKAE--KERANEPIFQAOLTRYACGGLVITGTACHQVADGQSMVFYITAW	167
HvTHT7	121	GMTTDVDGFYPSVPDPEESIGAALLQVKLSRYRCGGLLLGVICHHTIADGHAASTFYGAW	180
HvTHT8	121	GMTTDVDGFYPSVPDPEESIGAALLQVKLSRYRCGGLLLGVICHHTIADGHAASTFYGAW	180
HvTHT2	168	ASAVRTDS-AVLPSPFVDRSATVVRSPPKPAYDHRNTEFKG-ELSS-WSHSYGLPMDRI	224
HvTHT7	181	ATAVREGKGFIVPSPFIDRAATAVPRRTPKPVDFHRSIEFKGEGSGSSQSDALLPMDKI	240
HvTHT8	181	ATAVREGKGFIVPSPFIDRAATAVPRRTPKPVDFHRSIEFKGEGSGSSQSDALLPMDKI	240
HvTHT2	225	KNLAVTFPDEFVADLKARVGTIRCSTFQCLLAHWKKITAAARDLAPDDFTQVRVAVNCRGR	284
HvTHT7	241	KNITVNFTEAFMAELRSRVGARCSTFQCLLAHWKKMTAARGLSPEEFTQVRVAVNCRGR	300
HvTHT8	241	KNITVNFTEAFMAELRSRVGARCSTFQCLLAHWKKMTAARGLSPEEFTQVRVAVNCRGR	300
HvTHT2	285	AAPPVPMDFFGNNVLWAFPRMQVRDLSSSYPAVVGAIKDAVALVDEYHQSFIQFGEAE	344
HvTHT7	301	ANPPVSMDFFGNNVLWAFPRMQVRDLGLSYGGVVGAIKDAARIDDEYVQSFVDFG---	357
HvTHT8	301	ANPPVSMDFFGNNVLWAFPRMQVRDLGLSYGGVVGAIKDAARIDDEYVQSFVDFG---	357
HvTHT2	345	RGVIEDGGEELASTAATPGTMTCPDLEVDSWLGRFRHQLDFGCGPPCAFLPPDLPIEGIM	404
HvTHT7	358	-GVADANGEELEATS-TCGTMLCPDVEVDSWLGRFRHQLDFGTGPPSAFLPAGLPVEGMM	415
HvTHT8	358	-GVADANGEELEATS-TCGTMLCPDVEVDSWLGRFRHQLDFGTGPPSAFLPAGLPVEGMM	415
HvTHT2	405	IFVPSCDPKGCVDLFMALDDEHVQAFKQICYSMD-----	438
HvTHT7	416	VFVPSRTVKGSVDLFMALAEDHVAPFNKICYSLDDILPSRM	456
HvTHT8	416	VFVPSRTVKGSVDLFMALAEDHVAPFNKICYSLDDILPSRM	456

 Clade IV specific motif Clade IVb specific motif

Supplementary Figure S5. Amino acid sequences of HvTHT2, HvTHT7, and HvTHT8. The red and green squares indicate clade IV and IVb specific motifs, respectively. The HvTHT amino-acid sequences were obtained from the EnsemblPlants database (http://plants.ensembl.org/Hordeum_vulgare/Info/Index?db=core).



Supplementary Figure S6. Relationships between HvTHT proteins and HvTHT like proteins from various species. A dendrogram was generated from sequences of 115 HvTHT like proteins. Bootstrap values >70% (based on 1,000 replications) are indicated at each node (bar = 0.05 amino acid substitutions per site). Protein sequences

were obtained from GenBank (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) and IPK Rye Blast Server (<https://webblast.ipk-gatersleben.de/ryeselect/>). Abbreviation of species before the accession number were as follows: At (*Aegilops tauschii*), Bd (*Brachypodium distachion*), Do (*Dichanthelium oligosanthes*), Ec (*Eragrostis curvula*), Hv (*Hordeum vulgare*), Os (*Oryza sativa*), Ph (*Panicum hallii*), Pm (*Panicum miliaceum*), Sc (*Secale cereale*), Si (*Setaria italica*), Sv (*Setaria viridis*), Ta (*Triticum aestivum*), Td (*Triticum durum*), Tu (*Triticum urartu*), Zm (*Zea mays*). Accession number of OsTHTs OsTBTs were as follow: OsTHT1 (XP_015613139.1), OsTHT2 (XP_015612968.1), OsTBT1 (XP_015615935.1), and OsTBT2 (XP_015615816.1). The HvTHT amino-acid sequences were obtained from the EnsemblPlants database (http://plants.ensembl.org/Hordeum_vulgare/Info/Index?db=core). Red arrowheads indicate HvTHT2, HvTHT7, HvTHT8, OsTHT1, OsTHT2, OsTBT1, and OsTBT2.

Supplementary table S1. *HvTHT* genes detected by a search on the database, EnsemblPlants (http://plants.ensembl.org/Hordeum_vulgare/Info/Index?db=core).

Gene	Gene code	Similarity of amino acid sequece (%)			
		OsTBT1	OsTBT2	OsTHT1	OsTHT2
<i>HvTHT1</i>	HORVU1Hr1G019380	78.7	77.7	86.3	87.1
<i>HvTHT2</i>	HORVU1Hr1G019410.1	78.5	78.1	85.9	87.0
<i>HvTHT3</i>	HORVU2Hr1G125270.1	81.2	80.5	78.4	78.6
<i>HvTHT4</i>	HORVU2Hr1G125370.1	80.9	80.4	79.1	79.3
<i>HvTHT5</i>	HORVU4Hr1G077680.11	73.9	73.1	72.7	73.6
<i>HvTHT6</i>	HORVU4Hr1G077720.8	80.8	80.4	80.1	81.0
<i>HvTHT7</i>	HORVU4Hr1G077780.1	80.7	80.0	77.9	79.5
<i>HvTHT8</i>	HORVU4Hr1G077790	80.6	79.9	77.9	79.5
<i>HvTHT9</i>	HORVU6Hr1G073010.3	80.8	78.7	78.7	78.9

Supplementary table S2. Sequences of primers.

Name	Sequence (5'-3')
<i>qRT-PCR</i>	
<i>HvTHT1</i> -F	CATGTCCAGCAGCTACCCGA
<i>HvTHT1</i> -R	GCCAAAGTCAACAAACGACTG
<i>HvTHT2</i> -F	CCTGTCCAGCAGTTACCCAG
<i>HvTHT2</i> -R	CCCGAAGTCGATAAACGACTG
<i>HvTHT3</i> -F	CAGATTGACTTCGGCGCC
<i>HvTHT3</i> -R	GAGTAGAAGGTCGCCGTGTC
<i>HvTHT4</i> -F	Used Hvtht3 F
<i>HvTHT4</i> -R	CACAAGTAACGCGTGTCATGATC
<i>HvTHT5</i> -F	GGCTCATGATCTTCATGCCA
<i>HvTHT5</i> -R	CAAGGGATTCCGGTGGTGTC
<i>HvTHT6</i> -F	GAACGCATCGACGACGAGTA
<i>HvTHT6</i> -R	CTATCCACCTCTGCGTCCG
<i>HvTHT7/8</i> -F	GGATGATGGTCTTCGTGCC
<i>HvTHT7/8</i> -R	GCAAATCTTGTTGAATGGCG
<i>HvTHT9</i> -F	GATGCTGCGTACATCCAGTC
<i>HvTHT9</i> -R	AGACTGCTGTCCACCTCCAG
<i>ADP</i> -F	GCTCTCCAACAACATTGCCAAC
<i>ADP</i> -R	GAGACATCCAGCATCATTATTCC
<i>Cloning of HvTht</i>	
<i>HvTHT8/pGST</i> -F	CTGTTCCAGGGCCCGATGGAGGTTAACCACGCTGA
<i>HvTHT8/pGST</i> -R	TTCGGATCCCTCGAGTTACATCCTGGATGGGAGGA
<i>HvTHT2/pGST</i> -F	CTGTTCCAGGGCCCGATGGCAGTGATGGTGGAGAT
<i>HvTHT2/pGST</i> -R	TTCGGATCCCTCGAGTTAGTCCATTGAGTAGCAGATCTG
<i>HvTHT7/pGST</i> -F	CACCACCACATCGCCGACGGCCACGC
<i>HvTHT7/pGST</i> -R	GGCGATGTGGTGGTGGCATATCACGC
<i>pGST</i> inverse-F	CTCGAGGGATCCGAATTCAA
<i>pGST</i> inverse-R	CGGGCCCTGGAACAGAACTTC

Supplementary table S3. MRM conditions for triticamides and related compounds.

Compound	Precursor ion	Product ion	Cone voltage	Collision energy
	<i>m/z</i>	<i>m/z</i>	<i>V</i>	<i>eV</i>
Cinnamoyltryptamine	291.16	130.92	20	18
Cinnamoyl-9-hydroxy-8-oxotryptamine (1)	321.01	303.06	10	4
Cinnamoyl-8-oxotryptamine (2)	305.03	130.90	25	18
Cinnamoyl-(1 <i>H</i> -Indol-3-yl)methylamine (3)	277.06	129.98	20	10
Tryptamine	161.02	143.93	16	10
8-Oxotryptamine	175.07	157.13	15	10
(1 <i>H</i> -Indol-3-yl)methylamine	147.03	129.97	8	4
For detection of deuterium labeled phenylamides				
1 -phenyl- <i>d</i> 5	326.01	308.06	10	4
2 -phenyl- <i>d</i> 5	310.03	135.90	25	18