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Tri13 and *Tri7* Determine Deoxynivalenol- and Nivalenol-Producing Chemotypes of *Gibberella zeae*

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***Gibberella zeae*, a major cause of cereal scab, can be divided into two chemotypes based on production of the 8-ketotrichothecenes deoxynivalenol (DON) and nivalenol (NIV). We cloned and sequenced a *Tri13* homolog from each chemotype. The *Tri13* from a NIV chemotype strain (88-1) is located in the trichothecene gene cluster and carries an open reading frame similar to that of *Fusarium sporotrichioides*, whereas the *Tri13* from a DON chemotype strain (H-11) carries several mutations. To confirm the roles of the *Tri13* and *Tri7* genes in trichothecene production by *G. zeae*, we genetically altered toxin production in 88-1 and H-11. In transgenic strains, the targeted deletion of *Tri13* from the genome of 88-1 caused production of DON rather than NIV. Heterologous expression of the 88-1 *Tri13* gene alone or in combination with the 88-1 *Tri7* gene conferred on H-11 the ability to synthesize NIV; in the latter case, 4-acetylnivalenol (4-ANIV) also was produced. These results suggest that *Tri13* and *Tri7* are required for oxygenation and acetylation of the oxygen at C-4 during synthesis of NIV and 4-ANIV in *G. zeae*. These functional analyses of the *Tri13* and *Tri7* genes provide the first clear evidence for the genetic basis of the DON and NIV chemotypes in *G. zeae*.**

Gibberella zeae (Schwein.) Petch (anamorph: *Fusarium graminearum* Schwabe) is an important pathogen of cereal crops such as maize, wheat, and barley. *G. zeae* causes root rot and seedling diseases (6, 25), head blight of wheat and barley, and stalk and ear rot of maize (7, 21). Head blight and ear rot reduce grain yield, and the harvested grain often is contaminated with mycotoxins, such as trichothecenes and zearalenone. Cereals contaminated with trichothecenes are associated with feed refusal, vomiting, diarrhea, dermatitis, and hemorrhages in farm animals (26). Trichothecenes also appear to contribute to the virulence of *G. zeae* on host plants (29, 33).

G. zeae may be divided into two chemotaxonomic groups based on production of 8-ketotrichothecenes (16). The deoxynivalenol (DON) chemotype produces DON and acetyl-DONs such as 3-acetyl-DON (3-ADON) and 15-acetyldeoxynivalenol (15-ADON). The nivalenol (NIV) chemotype produces NIV and 4-acetyl-NIV (4-ANIV) (also known as fusarenon-X). The two chemotypes appear to differ in geographic distribution, with both DON and NIV chemotypes reported in several countries of Africa, Asia, and Europe (10, 15, 24, 36–38) but only the DON chemotype reported in North America (1, 30). DON and NIV are frequently found in cereals harvested in some Asian countries, e.g., Korea and Japan (18, 40, 42). NIV is present at higher levels than DON in cereals from these countries.

Trichothecenes are biosynthesized in a complex pathway involving a series of oxygenation, isomerization, and esterification steps, and the molecular genetics of T-2 toxin production by *Fusarium sporotrichioides* have been studied intensively (9, 14). Many of the trichothecene biosynthesis genes are localized in a gene cluster of at least 10 genes. The genes in this cluster include those for trichodiene synthetase (*Tri5*) (12), P450 oxygenase (*Tri4* and *Tri11*) (2, 13), acetyltransferase (*Tri3* and *Tri7*) (5, 28), transcription factors (*Tri6* and *Tri10*) (34, 39), a toxin efflux pump (*Tri12*) (3), and two unidentified hypothetical proteins (*Tri8* and *Tri9*) (14, 29). Another acetyltransferase gene (*Tri101*) (20) is unlinked to the cluster. Recently, two *F. sporotrichioides* genes, *Tri13* and *Tri14*, were found to be under the control of *Tri10* (39), but the functions of these genes are not known. Homologs of *Tri* genes have been reported for *G. zeae* (5, 19, 22, 27, 34, 39, 41).

In a previous study (22), we analyzed the sequences of *Tri* genes from *G. zeae* DON and NIV chemotypes. Of the 10 *Tri* gene homologs in the *Tri* gene cluster, all except *Tri7* were conserved; the *Tri7* open reading frame (ORF) is intact in NIV chemotypes, whereas it is defective in DON chemotypes. However, there has been no clear evidence for the genetic determinants of DON and NIV in *G. zeae*. Brown et al. (5) compared the sequences of the *Tri* gene clusters from *F. sporotrichioides* and *F. graminearum*. They reported that *Tri7* is required for acetylation of the oxygen on C-4 of the T-2 toxin in *F. sporotrichioides* and is nonfunctional in *F. graminearum*.

The general objective of this study was to better understand trichothecene biosynthesis by the two chemotypes of *G. zeae*. We reasoned that comparison of sequences within each trichothecene gene cluster would identify the determinants of DON and NIV production. The specific objectives of this study were (i) to sequence *Tri13* from DON and NIV producers, (ii) to study the functions of *Tri13* and *Tri7*, and (iii) to correlate differences in gene structure with differences in production of 8-ketotrichothecenes by the two chemotypes of *G. zeae*.

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MATERIALS AND METHODS

Strains, media, and culture conditions. *G. zeae* strains H-11 (a DON producer) and 88-1 (a NIV producer) were described previously (22) and were used

TABLE 1. PCR primers and plasmids used in this study

Primer or plasmid	Sequence (5′ to 3′) or characteristics[a]	GenBank accession no. (bp[b]) or reference
Primers		
Ntri7/p1	ACAGAACAGCGCGAATTGAGTCCA	AF336365 (3362–3385)
Ntri7/p2	AAAGATGATTCGGAGCCAGATGTTAGTA	AF336365 (6734–6707)
Tri7n/delp1	CCGGTGGGCCTAGTTTAAAGTTCAATCT	AF336365 (4429–4402)
Tri7n/delp2	CACTAAACTGAATCCTTGGCGAAAAAC	AF336365 (6400–6426)
Nwtri13/p1	GGCTGATAGGGCGGTCTTGAAAATGAAC	AY064209 (18–46)
Nwtri13/p2	CCTGGGAATTCAATGGTGTCAAGA	AY064209 (3660–3637)
Deltri13/p1	GAAGATCTACTTTGAGCTGTTGCCTTGTCCTA	AY064209 (853–830)[c]
Deltri13/p2	GAAGATCTGCCACAGCCACCAGACCGATAGAG	AY064209 (2505–2528)[c]
Plasmids		
pNTri7H	Fungal transformation vector (*HygB*^{R}, *Amp*^{R}, *Kan*^{R}) carrying an intact 88-1 *Tri7* ORF (9.4 kb)	This study
pdelN7H	Fungal transformation vector (*HygB*^{R}, *Amp*^{R}, *Kan*^{R}) carrying partial sequences of *Tri3* and *Tri8* used for deletion of *Tri7* via double crossover (9.4 kb)	This study
pNTri13G	Fungal transformation vector (*Gen*^{R}, *Amp*^{R}, *Kan*^{R}) carrying an intact 88-1 *Tri13* ORF (9.8 kb)	This study
pdelN13G	Fungal transformation vector (*Gen*^{R}, *Amp*^{R}, *Kan*^{R}) carrying partial 5′ and 3′ flanking sequences of *Tri13* used for deletion via double crossover (7.3 kb)	This study
pBCATPH	Plasmid carrying *Chr*^{R} and *HygB*^{R} (5.5 kb)	43
pBCGT	Plasmid carrying *Chr*^{R} and *Gen*^{R} (6.2 kb)	This study
pII99	Plasmid carrying *Gen*^{R} and *Amp*^{R} (5.3 kb)	31
pII99-1	pII99 derivative lacking the *Eco*RI site (5.3 kb)	This study

[a] *Amp*^{R}, resistant to ampicillin; *Kan*^{R}, resistant to kanamycin; *Chr*^{R}, resistant to chloramphenicol.
[b] The number in parentheses indicates the primer position in the deposited sequence.
[c] A *Bgl*II recognition site (AGATCT) was added to each 5′ end.

for sequence analysis and functional studies. Fungal strains from 25% glycerol stock cultures stored at −80°C were maintained on potato dextrose agar (Difco Laboratories, Detroit, Mich.). To isolate genomic DNA, fungal conidia were inoculated into 100 ml of complete liquid medium (8) at 10^6 per ml. Cultures were incubated at 25°C for 48 h on a rotary shaker (200 rpm), after which mycelia were harvested and lyophilized. Recombinant *Escherichia coli* strains were grown on Luria-Bertani agar (35) or liquid medium supplemented with 75 μg of ampicillin per ml.

DNA manipulation and PCR conditions. Fungal genomic DNA was prepared as previously described (17). *E. coli* colonies carrying recombinant plasmids were screened by a single-tube miniprep method (23). For sequencing, plasmids were purified from 5-ml *E. coli* cultures by using a kit from Qiagen Inc. (Valencia, Calif.). Standard procedures were used for restriction endonuclease digestions, ligations, gel blot analysis, and agarose gel electrophoresis (35). The PCR primers used in this study (Table 1) were obtained from the Bioneer oligonucleotide synthesis facility (Bioneer Corporation, Chungwon, Korea), dissolved at 100 μM in sterilized water, and stored at −20°C. PCRs were performed as described previously (44).

Amplification, cloning, and sequencing of *Tri13*. We amplified *Tri13* genes from the 88-1 and H-11 strains by using several sets of primers that were based on the known sequences of the *Tri12* gene from *G. zeae* and the *Tri13* and *Tri14* genes from *F. sporotrichioides* (39). Most of the primer pairs led to successful amplification of fragments of the expected sizes. These PCR products were sufficient for construction of contigs for both strains. PCR products of the expected sizes were cloned into pCR2.1TOPO by using a TOPO TA cloning kit (Invitrogen, San Diego, Calif.). Sequencing of the inserts in pCR2.1TOPO was initiated with M13 reverse and forward primers and then extended with specific primers corresponding to the newly sequenced regions. DNA sequencing was performed at the National Instrumentation Center for Environmental Management (Seoul National University, Suwon, Korea) with an ABI377 automated DNA sequencer (Applied Biosystems Inc., Foster City, Calif.). Primers for sequencing were designed by using the PrimerSelect program (DNASTAR, Inc., Madison, Wis.). Sequences were assembled using the SeqMan program (DNASTAR, Inc.) and analyzed with the MegAlign and MapDraw programs (DNASTAR, Inc.). BLAST (4) searches were performed against the National Center for Biotechnology Information and GenBank databases.

Plasmid construction. For insertion of *Tri7* from 88-1 into the genome of H-11, a plasmid containing an intact copy of *Tri7* was prepared. *Tri7* was amplified from 88-1 genomic DNA by using primers Ntri7/p1 and Ntri7/p2 and cloned into pCR2.1TOPO as described above. The *Tri7* insertion vector pNTri7H (9.4 kb) was then created by subcloning a 2.1-kb hygromycin B resistance gene (*HygB*^{R}) taken from pBCATPH into the *Xba*I site of the pCR2.1TOPO-*Tri7* vector described above. For deletion of *Tri7* from the genome of 88-1 via double crossover, a plasmid harboring a 3.4-kb fragment carrying the 5′ and 3′ flanking sequences of the 88-1 *Tri7* ORF was constructed. This fragment, which carried 0.9 and 2.5 kb of the 5′ and 3′ flanking sequences, respectively, was obtained by inverse PCR with primers Tri7n/delp1 and Tri7n/delp2, using *Nhe*I-digested and self-ligated 88-1 genomic DNA as a template. The inverse PCR product was cloned into pCR2.1TOPO along with the *HygB*^{R} cassette, creating a 9.4-kb plasmid designated pdelN7H.

Plasmids used for insertion or deletion of the 88-1 *Tri13* gene were prepared by the same strategies described above for *Tri7*. For an insertion plasmid, primers NWTri13/p1 and NWTri13/p2 were used to amplify the entire *Tri13* ORF from 88-1 genomic DNA. The 3.6-kb amplified product was cloned into pCR2.1TOPO and then removed from the cloning vector by digestion with *Eco*RI. This *Eco*RI fragment was subcloned into an *Eco*RI site of pBCGT, which contains a gene conferring resistance to Geneticin (*Gen*^{R}), to create the 9.8-kb plasmid pNTri13G. To create a deletion plasmid, 88-1 genomic DNA was digested with *Eco*RI, self-ligated, and used as a template for inverse PCR with primers delTri13/p1 and delTri13/p2, each including a *Bgl*II recognition sequence. The PCR amplified a 2.0-kb fragment carrying the 5′ and 3′ flanking sequences of the 88-1 *Tri13* gene. The amplified product was cleaned by phenol extraction, digested with *Bgl*II, and ligated into *Bgl*II-digested pII99-1 containing *Gen*^{R} and no *Eco*RI site. The resulting 7.3-kb plasmid was designated pdelN13G (Table 1).

Fungal transformation. For sporulation, mycelial plugs of each strain were inoculated into CMC liquid medium (15 g of carboxylmethyl cellulose, 1 g of yeast extract, 0.5 g of $MgSO_4$, 1 g of NH_4NO_3, and 1 g of KH_2PO_4 per liter) at 25°C with shaking (100 rpm) for 3 days. Fungal conidia produced in CMC culture were inoculated into 100 ml of YPG liquid medium (3 g of yeast extract, 10 g of peptone, and 20 g of glucose per liter) at 10^6 per ml and grown for 12 h with shaking at 25°C. Mycelia were harvested by filtration through sterile Whatman no. 2 filter paper and incubated in 80 ml of 1 M NH_4Cl containing Driselase (10 mg/ml) (InterSpex Products, Inc., San Mateo, Calif.) to generate protoplasts. Further steps in transformation were as previously described (45). Each transformant was transferred to fresh potato dextrose agar medium amended with the desired antibiotics and purified by single-conidium isolation. For insertion of the 88-1 *Tri7* or *Tri13* gene, circular pNTri7H or pNTri13G was transformed into protoplasts of H-11. For gene deletions, plasmids pdelN7H and pdelN13G were linearized by digestion with *Nhe*I and *Eco*RI, respectively, prior to transformation.

Trichothecene analysis. Transgenic and wild-type strains of *G. zeae* were screened for trichothecene production on rice medium. Rice cultures were harvested after 3 weeks of incubation at 25°C and extracted as previously described (36). A portion of each extract was reacted with trimethylsilating reagent and analyzed with a JEOL JMS-AX 505 gas chromatograph-mass spectrometer in full-scan mode using a DB-5 fused silica column (0.25 mm [inside diameter] by 30 m; 0.25-μm film) (J & W Scientific, Folsom, Calif.). The column temperature was maintained at 120°C for 5 min and then increased to 270°C at 5°C per min.

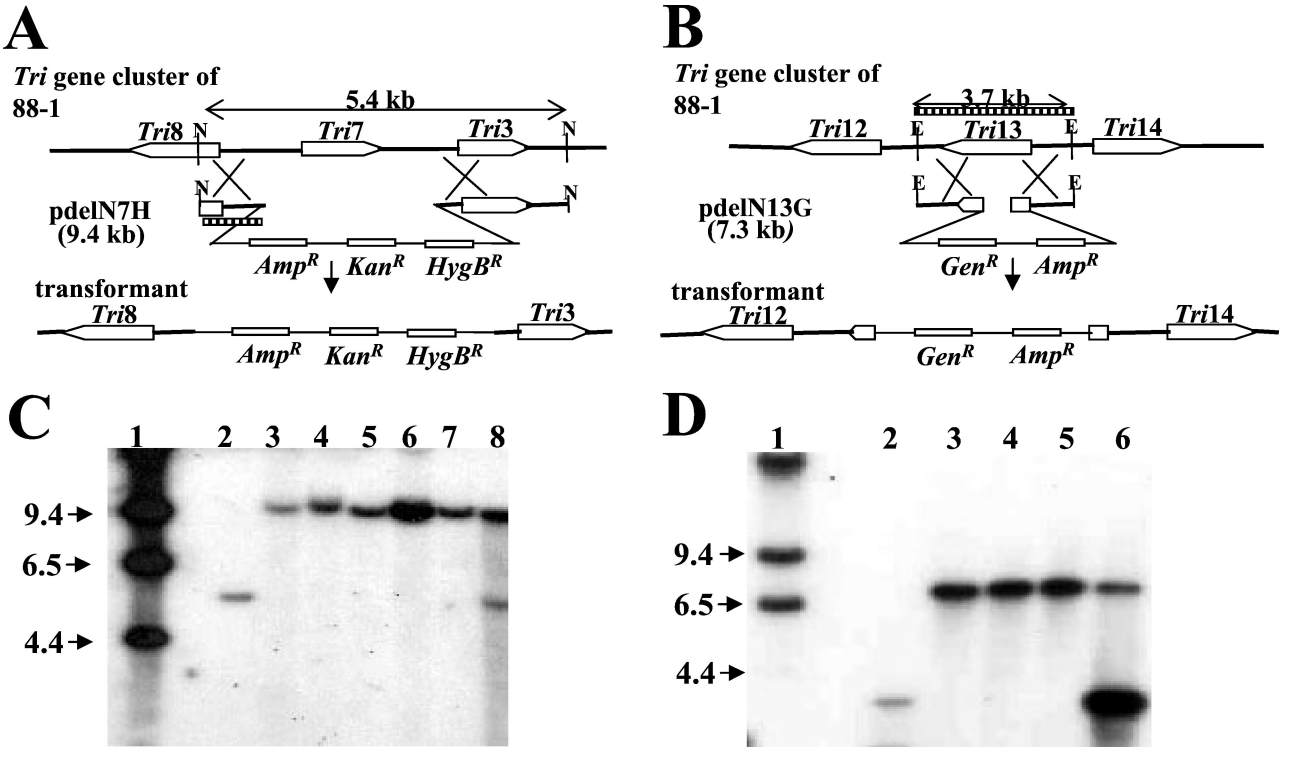

FIG. 1. Schemes for deletion of either *Tri7* or *Tri13* from the genome of *G. zeae* 88-1 (A and B) and gel blots of genomic DNAs from *Tri7* (C) and *Tri13* (D) deletion transformants digested with *Nhe*I and *Eco*RI, respectively. (A and B) Probes used for hybridization are marked as cross-hatched. N, *Nhe*I; E, *Eco*RI; Amp^R, ampicillin resistance gene; Kan^R, kanamycin resistance gene; $HygB^R$, hygromycin B resistance gene; Gen^R, geneticin resistance gene. (C) Lane 1, lambda DNA cut with *Hin*dIII; lane 2, wild-type 88-1; lanes 3 to 7, *Tri7* deletion strains of 88-1, TxNΔ7-1, TxNΔ7-2, TxNΔ7-3, TxNΔ7-4, and TxNΔ7-5, respectively; lane 8, transformant TxNΔ7-6 carrying the transforming vector at an ectopic site. (D) Lane 1, lambda DNA cut with *Hin*dIII; lane 2, wild-type 88-1; lanes 3 to 5, *Tri13* deletion strains of 88-1 , TxNΔ13-1, TxNΔ13-2, and TxNΔ13-3, respectively; lane 6, transformant TxNΔ13-4 carrying the transforming vector at an ectopic site. Band sizes in kilobases are marked with arrows on the left.

The injector, ion source, and interface temperatures were 280, 200, and 250°C, respectively. The ionizing voltage was 70 eV.

Nucleotide sequence accession numbers. The sequences of the *Tri13* genes obtained from *G. zeae* 88-1 and H-11 have been deposited in GenBank under accession numbers AY064209 and AY064210, respectively.

RESULTS

Comparative sequence analysis of *Tri13*. The putative ORF of a *Tri13* homolog from *G. zeae* strain 88-1 was identified by sequence comparison with the cDNA sequence of *Tri13* from *F. sporotrichioides* (GenBank accession number AF330109). The *Tri13* ORF is located in the trichothecene gene cluster of *G. zeae* 88-1 immediately upstream of *Tri12*, as it is in *F. sporotrichioides*. This 1,853-bp ORF is interrupted once by a putative intron of 62 bp. The *Tri13* genes from 88-1 and *F. sporotrichioides* are 78 and 80% identical at the nucleotide and amino acid levels, respectively; both exhibit similarities to a putative cytochrome P450 monooxygenase. Seventeen amino acids at the N terminus of the *F. sporotrichioides* TRI13 protein are missing in the corresponding region of the putative TRI13 protein of 88-1 (data not shown).

G. zeae strain H-11 carries a *Tri13* homolog that is strikingly different from the *Tri13* homologs of 88-1 and *F. sporotrichioides*. The H-11 *Tri13* gene is only 65 and 61% identical to the *Tri13* genes from *G. zeae* 88-1 and *F. sporotrichioides*, respectively. In addition, alignment of these nucleotide sequences reveals many alterations present only in the H-11 gene. This gene appears to have incurred several substitutions, insertions, and deletions, causing a deficient translation start and frameshifts in the putative TRI13 amino acid sequence. Nucleotide alignment of *Tri13* fragments amplified from genomic DNAs of other *G. zeae* isolates revealed that these features found in H-11 *Tri13* are highly conserved among DON-producing isolates of the *G. zeae* strains tested from Korea and the United States (data not shown).

Molecular manipulations of *Tri7* and *Tri13*. (i) Deletion of either the *Tri7* or *Tri13* ORF from the *G. zeae* genome. The region containing the entire *Tri7* ORF or an internal portion of the *Tri13* ORF in the genome of strain 88-1 was deleted by targeted gene replacement using linearized pdelN7H and pdelN13G, respectively, via double crossover between homologous regions (Fig. 1A and B). The desired transformants sustaining a deletion of either *Tri7* or *Tri13* were identified by gel blot analysis (Fig. 1C and D). In transformants TxNΔ7-2, TxNΔ7-3, TxNΔ7-4, and TxNΔ7-5, a single 9.4-kb band (in lieu of a 5.4-kb native band) was observed to hybridize with a *Tri7* probe, indicating that a 2.0-kb region including the entire *Tri7* ORF was deleted and replaced with the vector pdelN7H (Fig. 1A and C). The transformants TxNΔ13-1, TxNΔ13-2, and TxNΔ13-3 contained the intact *Tri7* ORF but carried a 520-bp deletion of the *Tri13* ORF, which was replaced with the vector pdelN13G (Fig. 1B and D).

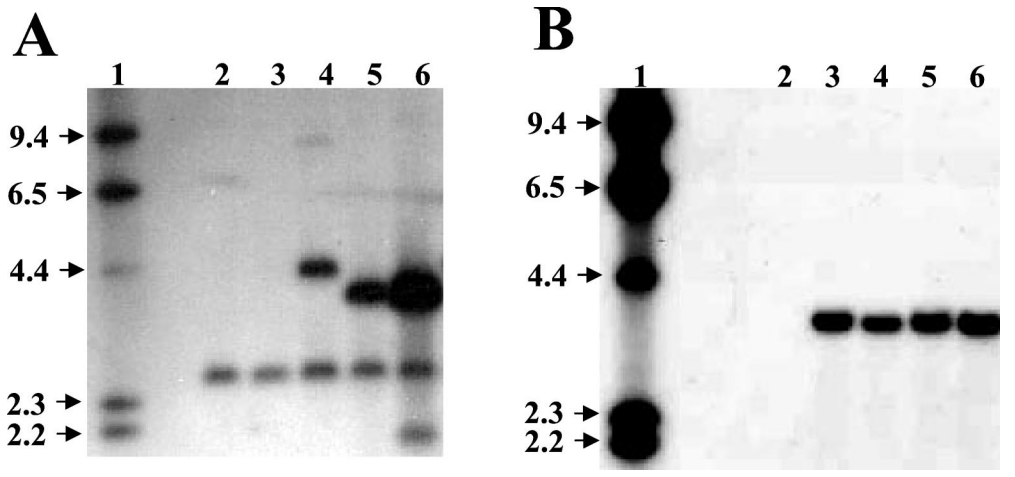

FIG. 2. DNA gel blots of *Eco*RI-digested genomic DNAs from *G. zeae* H-11 transformants carrying heterologous *Tri13* alone (A) or both *Tri13* and *Tri7* (B). (A) A 3.6-kb *Eco*RI fragment containing the entire *Tri13* ORF of 88-1 was used as a probe. Lane 1, lambda-*Hin*dIII markers; lanes 2 and 3, wild-type H-11 showing a 2.5-kb native *Tri13* band; lane 4, transformant TxN13 containing a truncated copy of the 88-1 *Tri13* gene; lanes 5 and 6, transformants TxN13-2 and TxN13-3, each carrying an intact copy of the 88-1 *Tri13* gene. (B) Transformants carrying both the 88-1 *Tri13* and *Tri7* genes were obtained by retransformation of TxN13-2 with pNTri7H. The probe was a 3.4-kb PCR product containing the entire *Tri7* gene of 88-1. Lane 1, lambda-*Hin*dIII markers; lane 2, wild-type H-11; lanes 3 to 6, TxN713-1, TxN713-2, TxN713-3, and TxN713-4, respectively (transformants carrying an intact copy of the 88-1 *Tri7* gene). Band sizes in kilobases are marked with arrows on the left.

(ii) Heterologous expression of intact *Tri7* and/or *Tri13* ORFs. The DON-producing H-11 strain of *G. zeae* was transformed with the circular vectors pNTri7H and pNTri13G, either singly or in sequential combination (Table 1). Vector pNTri7H carries the *Tri7* ORF from 88-1 and the $HygB^R$ gene; vector pNTri13G carries the *Tri13* ORF from 88-1 and the Gen^R gene. The resulting $HygB^R$ Gen^S, $HygB^S$ Gen^R, or $HygB^R$ Gen^R transformants were purified by single-conidium isolation, and integration events were examined by gel blot analysis.

The probe, a 3.6-kb *Eco*RI fragment from pNTri13G carrying the 88-1 *Tri13* ORF, hybridized with genomic DNAs from all $HygB^S$ Gen^R transformants tested. In *Eco*RI-digested genomic DNAs of transformants TxN13-2 and TxN13-3, the 3.6-kb fragment of the heterologous *Tri13* ORF hybridized along with a 2.5-kb fragment of the native *Tri13*, indicating that the intact 88-1 *Tri13* ORF integrated at an ectopic site of the recipient genome (Fig. 2A). In *Eco*RI-digested genomic DNAs from all $HygB^R$ Gen^S transformants tested, a 3.4-kb probe carrying the 88-1 *Tri7* ORF hybridized with a single 3.6-kb fragment of the heterologous *Tri7* but not with a native *Tri7*, probably because the probe shared only 80% nucleotide identity with the native *Tri7* (data not shown). The same probe identified $HygB^R$ Gen^R transformants carrying both intact 88-1 *Tri7* and *Tri13* ORFs at ectopic sites that were created by retransformation of transformant TxN13-2 with pNTri7H (Fig. 2B).

Trichothecene production by transgenic strains. (i) Transgenic *G. zeae* 88-1 strains with either *Tri7* or *Tri13* deleted. *G. zeae* 88-1 produced NIV and 4-ANIV in rice cultures (Fig. 3A), whereas all of the transgenic 88-1 strains tested that had sustained a deletion of the *Tri7* ORF produced NIV but no 4-ANIV (Fig. 3C). In contrast, other 88-1 strains resulting from ectopic integration of the transforming vector (pdelN7H) produced NIV and 4-ANIV similarly to the wild-type strain (88-1) (Fig. 3D).

Transgenic strains of 88-1 with targeted deletions of the *Tri13* ORF from the 88-1 genome exhibited more dramatic changes in trichothecene production. All of the transgenic strains tested that had sustained deletions of the *Tri13* ORF produced neither NIV nor 4-ANIV. Instead, they produced DON (Fig. 3E). Other strains resulting from ectopic integrations of pdelN13G produced NIV and 4-ANIV similarly to wild-type 88-1 (Fig. 3F).

(ii) Transgenic *G. zeae H-11 strains carrying heterologous Tri7* and/or *Tri13* ORFs. *G. zeae* H-11 produced DON and 15-ADON in rice cultures (Fig. 3B). Transgenic *G. zeae* strains of H-11 carrying intact copies of the 88-1 *Tri7* ORF produced DON and 15-ADON similarly to wild-type H-11 (data not shown). However, heterologous expression of the 88-1 *Tri13* ORF in the genetic background of H-11 caused changes in trichothecene production. GC-MS analysis showed that transgenic H-11 strains carrying the 88-1 *Tri13* ORF produced only NIV; neither 4-ANIV nor DON was produced (Fig. 3G). Furthermore, transgenic H-11 strains carrying intact copies of both the 88-1 *Tri7* and *Tri13* ORFs produced both NIV and 4-ANIV (Fig. 3H).

DISCUSSION

We located the *Tri13* gene in the trichothecene gene cluster and found that the gene differs drastically between two chemotypes of *G. zeae*. In *G. zeae* strain H-11, several mutations are present in the nucleotide sequence of *Tri13*. These mutations were found in all DON-producing *G. zeae* field isolates tested, suggesting that the lack of a viable *Tri13* gene is specific to the DON chemotype. Taken together with previous results concerning *Tri7* (22), these results suggest that both the *Tri7* and *Tri13* genes are nonfunctional in all DON chemotypes. In a previous experiment, disruption of *Tri7* in *F. sporotrichioides* caused accumulation of HT-2 toxin rather than T-2 toxin in fungal liquid culture, suggesting that *Tri7* is required for acet-

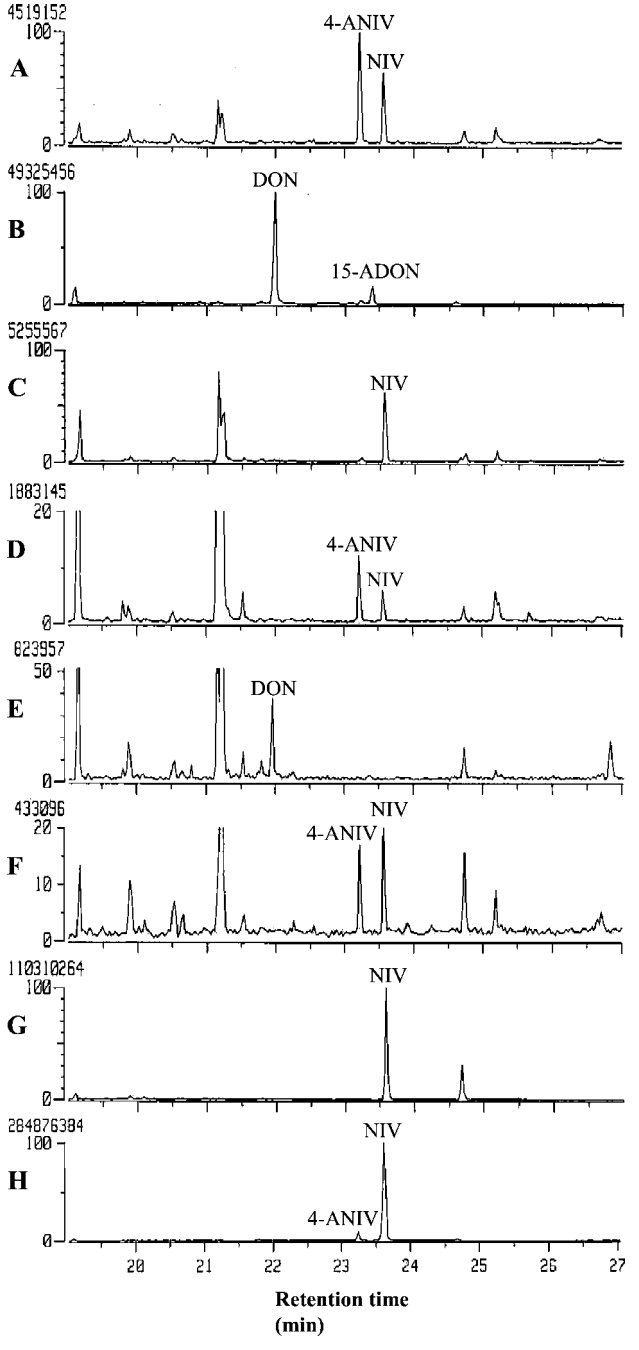

FIG. 3. Total ion chromatograms of extracts of *G. zeae* cultures. (A) Wild-type 88-1; (B) wild-type H-11; (C) transgenic 88-1 with *Tri7* deleted (TxNΔ7-1); (D) transgenic 88-1 carrying an ectopic integration of pdelN7H (TxNΔ7-6); (E) transgenic 88-1 with *Tri13* deleted (TxNΔ13-1); (F) transgenic 88-1 carrying an ectopic integration of pdelN13G (TxNΔ13-4); (G) transgenic H-11 carrying *Tri13* from 88-1 (TxN13-2); (H) transgenic H-11 carrying both *Tri13* and *Tri7* from 88-1 (TxN713-1).

ylation of the hydroxyl group at C-4 of the *F. sporotrichioides* T-2 toxin (5).

The functions of *Tri7* and *Tri13* in trichothecene production by *G. zeae* have not yet been conclusively determined. To confirm their functions, we employed molecular manipulations, including gene deletion and insertions, as described in this study. When the *Tri13* gene in the NIV-producing *G. zeae* strain 88-1 was deleted, DON, instead of NIV, was detected in rice cultures. This result indicates that the TRI13 protein is responsible for the oxygenation at C-4 during synthesis of NIV. In addition, heterologous expression of the functional copy of *Tri13* in the genetic background of the DON chemotype H-11 showed that *Tri13* is sufficient for conversion from DON production to NIV production in transgenic H-11 strains.

The same molecular strategies were used to confirm that the *G. zeae* TRI7 protein is involved in acetylation of the oxygen at C-4 of NIV, as in *F. sporotrichioides* (5). Heterologous expression of both the *Tri13* and *Tri7* genes in H-11 caused production of both NIV and 4-ANIV. Therefore, these functional analyses have confirmed that the *Tri13* gene is the determinant for the DON-NIV switching in *G. zeae* and that the *Tri7* gene is responsible for further modification of NIV. Further confirmation of the enzymatic activities of these proteins and their roles in trichothecene biosynthesis awaits detailed biochemical studies.

Transgenic strains produced toxins in quantities similar to those produced by recipient strains, although the kinds of trichothecenes were switched with respect to each other. H-11 produced approximately 5 times more trichothecenes than did 88-1. Transgenic H-11 carrying the 88-1 *Tri13* gene produced more NIV than did wild-type 88-1. The amount of DON produced by transgenic 88-1 with *Tri13* deleted was much less than that produced by the wild-type H-11. Studies of field isolates also revealed higher levels of DON production by DON chemotypes than of NIV production by NIV chemotypes (36). This difference may be attributed to differences in regulatory factors such as the *Tri6* and *Tri10* genes (34, 39) and/or in another quantitative genetic element(s).

In addition to functional studies, the transgenic strains created in this study will be useful in evaluating the relative contributions of the two types of trichothecenes in *G. zeae* pathogenesis toward cereals. Previous studies using a DON-deficient *G. zeae* mutant showed that DON was responsible for reduced virulence by *G. zeae* toward wheat (29, 33). However, the role of NIV in the virulence of *G. zeae* has not been quantitatively analyzed, although NIV is known to be less phytotoxic than DON (11). Combinations of isogenic strains differing only in trichothecene production, such as 88-1 and a transgenic 88-1 *Tri13* deletion strain or H-11 and a transgenic H-11 strain carrying the 88-1 *Tri13* gene, would be appropriate for these studies.

All DON chemotype isolates of *G. zeae* examined thus far carry defective sequences for both *Tri7* and *Tri13*, which raises two questions regarding the population of *G. zeae* in Korea. First, is the presence of both defective genes in the trichothecene gene clusters common to all DON-producing isolates? Based on our functional studies, which suggest that *Tri7* is not directly involved in DON-NIV switching, we can expect to find NIV- and DON-producing isolates of *G. zeae* that carry functional *Tri13* but not *Tri7* genes and functional *Tri7* but not *Tri13* genes, respectively. In the former case, the isolate should not be able to produce 4-ANIV because it has a defective *Tri7* gene. The latter case may be rare, because a functional *Tri7* gene may be dispensable, and thus mutations could accumulate. A PCR assay using primers derived from *Tri7* (22) and *Tri13* designed to reveal polymorphisms between the two che-

motypes would be useful in testing these possibilities. This PCR assay would also provide a more reliable method to determine the chemotypes of *G. zeae* field isolates.

The second question is whether polymorphisms in *Tri7* and *Tri13* between DON and NIV chemotypes reflect the genetic diversity of Korean *G. zeae* populations. No polymorphisms were found in the gene clusters including *Tri7* and *Tri13* among the Korean NIV-producing isolates tested (unpublished data). In contrast, in the DON-producing isolates, the gene clusters differed at the two *Tri* genes. Significant conservation of this structural difference between the *Tri* gene clusters in the two chemotypes would suggest the presence of chemotype-specific lineages in Korea. According to the description by O'Donnell et al. (32), a preliminary study showed that Korean *G. zeae* populations from barley were dominated by a single lineage (lineage 6) and that those from maize were dominated by lineage 7, but lineage 3 was a relatively common component (K. A. Zeller, J. I. Vargas, Y.-W. Lee, R. L. Bowden, and J. F. Leslie, Abstr. National Fusarium Head Blight Forum, abstr. 163, 2001). It is likely that at least lineages 6 and 7 in Korean populations are specific to the NIV and DON chemotypes, respectively. Confirmation of this hypothesis will require a phylogenetic study of Korean *G. zeae* populations.

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