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# Patterns of Trichothecene Production, Genetic Variability, and Virulence to Wheat of *Fusarium graminearum* from Smallholder Farms in Nepal

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*Fusarium graminearum* causes wheat head blight and contaminates grain with the trichothecenes 4-deoxynivalenol and nivalenol. Sequence analysis of trichothecene genes indicates that nivalenol production is the ancestral trait; however, deoxynivalenol producers occur worldwide and predominate in North and South America and in Europe. Analysis of a large field population (>500 strains) from Nepal identified three groups that were both genetically distinct and polymorphic for trichothecene production: SCAR1 comprising 95% deoxynivalenol producers, SCAR2 comprising 94% nivalenol producers, and SCAR3/5 comprising 34% deoxynivalenol producers/63% nivalenol producers. The ability to cause wheat head blight differed between SCAR groups and trichothecene chemotypes: deoxynivalenol producers were more virulent than nivalenol producers across all three SCAR groups and within the SCAR3/5 genetic background. These data support the hypothesis that production of deoxynivalenol rather than nivalenol confers a selective advantage to this important wheat pathogen.

**KEYWORDS: Trichothecenes; *Fusarium*; wheat head blight; *Triticum aestivum***

## INTRODUCTION

*Fusarium graminearum* (sexual stage *Gibberella zeae*) is a major cause of head blight of wheat (*Triticum aestivum*) and ear rot of maize (*Zea mays*) worldwide (*1*). *F. graminearum* not only reduces yield but also contaminates grain with trichothecene toxins that cause mycotoxicoses in humans and animals. The trichothecenes comprise a large family of sesquiterpene 12,13-epoxides with potent activity against ribosomal protein synthesis (*2*). In *Fusarium*, trichothecenes are synthesized by a complex biosynthetic pathway that requires the coordinated expression of more than 14 trichothecene (*TRI*) genes (*3*). In *F. graminearum*, the ultimate product of the pathway is nivalenol (NIV), which has hydroxyl groups at carbon positions 3 (C-3), C-4, C-7, and C-15 and a keto group at C-8; 4-deoxynivalenol (DON) is a pathway intermediate (**Figure 1**) (*4*). Many strains of *F. graminearum*, however, are unable to hydroxylate the C-4 position and accumulate DON rather than NIV.

Gene disruption and complementation experiments have shown that one gene, *TRI13*, is sufficient to convert a strain from DON to NIV production (*4*). NIV producers have a functional copy of the *TRI13* gene, which encodes a cytochrome P450 monooxygenase that is required for oxygenation at C-4. In contrast, all DON producers studied to date have a nonfunctional copy or pseudogene of *TRI13* and, in addition, a nonfunctional or deleted copy of the *TRI7* gene, which encodes a 4-acetyltransferase that converts NIV to 4-acetylnivalenol (*4*–*9*). In *F. graminearum*, *TRI7* and *TRI13* genes and pseudogenes are located in a cluster of 12 contiguous trichothecene biosynthetic genes. Functional copies of *TRI7* and *TRI13* genes are similarly clustered in the related species *Fusarium sporotrichioides*, which produces T-2 toxin and other trichothecenes that are hydroxylated and acetylated at C-4 (*5*, *7*). These DNA sequence data support the conclusion that DON biosynthesis

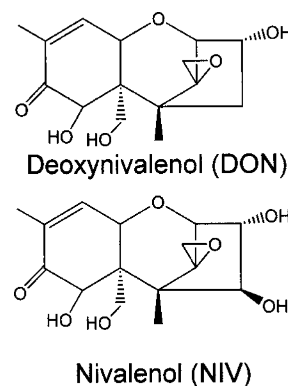


**Figure 1.** Structures of DON and NIV.

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in *F. graminearum* was derived from the NIV biosynthetic pathway by loss-of-function mutations.

Trichothecene management strategies include collection and analysis of samples to determine the occurrence and level of contamination of cereal grains and processed foods. Therefore, the natural occurrence of DON, NIV, and other trichothecenes in cereal grains has been surveyed worldwide using rigorous analytical chemical methods such as high-performance liquid chromatography and gas chromatography−mass spectrometry (GC-MS). To date, surveys of trichothecenes in wheat and other cereal grains infected with *F. graminearum* indicate that DON predominates in North America (*10*, *11*), South America (*12*, *13*), and Europe (*12*, *14*, *15*). NIV contamination is rare in cereal grains in North and South America, although it is somewhat more frequent in Europe. In contrast, surveys in China, Japan, Korea, and Nepal have found both DON and NIV as frequent cocontaminants of grain infected with *F. graminearum* (*16*−*19*). Moreover, both DON-producing and NIV-producing strains of *F. graminearum* have been isolated from wheat and other cereal grains in Asia (*4*, *6*, *7*, *19*). Thus, although DNA sequence analysis indicates that NIV production is the ancestral trait, the worldwide distribution of DON and of DON-producing strains of *F. graminearum* today suggests that DON production may have some selective advantage for this important wheat pathogen.

The main objective of this study was to investigate the relationship between trichothecene chemotype and virulence of *F. graminearum* on wheat. The approach was to characterize patterns of trichothecene production, genetic variability, and virulence to wheat in a large field population (>500) of strains from one location in Nepal. Although several recent studies have reported a high level of genetic variability of *F. graminearum* in the United States and in Asia (*9*, *20*−*24*), none of these studies have incorporated chemical analysis of trichothecene production and analysis of virulence in a large field population of strains. Such a population genetics analysis should facilitate correlation of trichothecene chemotypes and genetic markers with plant pathogenesis and other traits of agricultural importance.

## MATERIALS AND METHODS

**Plant Sample Collection.** Plant samples were collected during the months of February to June in 1993, 1997, and 2000 from smallholder farms in the Lamjung district in central Nepal. The samples were collected in Gau Shahar village on the northern slope and in Purankot village on the southern slope of a mountain ridge at elevations between 1200 and 1600 m above sea level (latitude 28° 25′ N, longitude 84° 9′ E). The total collection area was approximately 20 $km^2$. *F. graminearum* strains were isolated from maize, wheat, rice, soil debris, and weeds. The sample collection methods in 1993 and 1997 were previously described (*19*, *25*).

Maize seed samples were collected from individual farms in Gau Shahar village in 1993 (two farms), 1997 (one farm), and 2000 (three farms) and in Purankot village in 2000 (three farms). All maize samples were locally grown cultivars. Maize seeds with visible symptoms of rot were selected from individual ears or from loose seeds if ears were not available on the farm. Wheat seed samples were collected from individual farms in Gau Shahar village (12 farms) and Purankot village (three farms) in 1997. Because of winter drought conditions in 2000, wheat samples were not available for that year. Three 1997 wheat samples were a small-seeded landrace of wheat, and the rest were improved wheat cultivars. Rice seed samples with husks were collected from individual farms in Gau Shahar village in 1997 (seven farms) and 2000 (two farms) and in Purankot village in 1997 (one farm) and 2000 (three farms). Wheat and rice seeds were not selected for disease symptoms and were chosen randomly for strain isolation.

Soil debris and weed samples were collected from small terraced fields that were fallow at the time of sampling in March 2000. The debris samples were collected from soils of five fields from each of two farms in Gau Shahar village and two farms in Purankot village. The weed samples were collected from the uncultivated borders and terrace walls of the same fields. On the basis of farmer interviews, the cropping history in 1999 was maize followed by rice in the fields in Purankot village and various vegetables and grains in Gau Shahar village. The soil debris components varied with the previous crop, containing identifiable rice roots and maize stalk segments, as well as a variety of unidentified plant roots and leaves. The weed samples contained a variety of plant leaves, stems, flowers, and seeds, but the plant species were not identified. At the farms, pieces of debris were placed on a sieve, washed with spring water to remove soil, and air-dried.

The transport of plant materials from Nepal to the United States and all research on Nepalese strains of *F. graminearum* were conducted under permit from the U.S. Department of Agriculture, Plant Health and Inspection Service.

**Isolation and Identification of *F. graminearum*.** Seeds of maize, wheat, and rice were surface disinfested with 0.5% sodium hypochlorite and then placed on a *Fusarium* selective medium containing pentachloronitrobenzene (*26*), and no more than one strain per seed was purified by single spore isolation and identified by morphology as *F. graminearum* (*26*). Pieces of debris and weeds, less than two cm in length, were placed on selective medium, and *F. graminearum* strains were isolated as described above.

**Genetic Analyses.** Five hundred seventy-six strains identified by morphology as *F. graminearum* were confirmed and characterized for genetic diversity using a species specific, SCAR polymorphism. DNA was extracted using an Ultraclean DNA Purification Kit (Mo Bio Laboratories, Inc., Solana Beach, CA) from the lyophilized mycelium of 7−10 day old cultures grown on V-8 juice agar. PCR amplification using primer pair Fg 16F/R was carried out as described (*21*, *22*), and the products were separated by electrophoresis through a 2% agarose gel, stained with ethidium bromide, and viewed under UV light. To distinguish between SCAR types 2 and 5, which differ at only five nucleotides, PCR-generated DNA was digested with *Fnu* 4H1 (New England BioLabs, Inc., Beverly, MA) prior to electrophoresis through a 1% agarose gel. *Fnu* 4H1 does not cut SCAR5 but does cut SCAR2 at one site to generate fragments of approximately 364 and 194 nt. The size of PCR products was estimated by comparison with known DNA standards.

Allelism of SCAR markers and the location of the SCAR locus on the genetic map of *F. graminearum* were determined by PCR amplification of 96 progeny and the parents of a previously described cross between strain GZ3639 from wheat in Kansas, United States (*27*), and R-5470 from barley in Japan. Genetic mapping of the trait was performed using Map Manager QTX11 as previously described (*28*).

One hundred forty strains were analyzed using AFLP analysis. Procedures developed by Zeller et al. (*24*) were followed, except that a fluorescent dye label was used instead of a radioactive label and final separation of the amplification products was conducted at the Gene Sequencing Facility, Michigan State University (East Lansing, MI). In brief, initial digests were performed using the restriction enzymes EcoR1 and Mse I at 37 °C for 2 h. Selective amplification was done using the Mse1-CA specific primer and the fluorescent-labeled *EcoR*I-AA primer (Applied Biosystems, Foster City, CA).

**Virulence Analysis.** Two hundred forty-six strains were scored for virulence on wheat heads in nine greenhouse tests as previously described (*29*). The susceptible cultivar Wheaton, which was developed in the United States, was used for routine virulence testing of all *F. graminearum* strains in eight tests. A small-seeded landrace of wheat obtained from Lamjung, Nepal, in 2000 was used for additional virulence testing of 37 fungal strains in one test. In brief, wheat plants were inoculated at anthesis by injecting a drop of mung bean medium containing 1000 macroconidia into one floret of a central spikelet of each head. Each strain was tested once, and a total of 10 heads were inoculated for each treatment. A high virulence, DON-producing strain GZ3639 was included as a standard strain in each greenhouse test. Disease severity was assessed as percentage diseased spikelets per head. Each individual head was scored for head blight four times after inoculation, usually at 7, 12, 18, and 21 days after inoculation. The

**Table 1.** Number of *F. graminearum* Strains from Each Plant Host in Relation to SCAR Markers and Trichothecene Chemotypes

| | SCAR marker[a] | | | | | chemotype | | | |
|---|---|---|---|---|---|---|---|---|---|
| plant host | 1 | 2 | 3/5 | 4 | total | DON | NIV | none | total |
| maize | 6 | 75 | 63 | 0 | 144 | 16 | 47 | 4 | 67 |
| wheat | 2 | 9 | 64 | 0 | 75 | 22 | 45 | 3 | 70 |
| rice | 13 | 31 | 78 | 2 | 124 | 20 | 38 | 0 | 58 |
| weeds | 6 | 13 | 36 | 0 | 55 | 10 | 21 | 0 | 31 |
| debris | 20 | 64 | 93 | 1 | 178 | 10 | 25 | 0 | 35 |
| all hosts | 47 | 192 | 334 | 3 | 576 | 78 | 176 | 7 | 261 |

[a] SCAR markers were scored by PCR analysis of fungal DNA as previously described (*21*). Trichothecene chemotypes were scored by GC-MS analysis of infected wheat heads or laboratory culture material as previously described (*22*, *28*).

formula of Shaner and Finney (*30*) was used to produce an AUDPC rating for disease development on each head. The heads were harvested at maturity and threshed individually by hand. The kernels were weighed and stored at −20 °C.

**Trichothecene Analysis.** For trichothecene analysis, kernels from 10 replicate heads of each treatment were pooled, ground, and analyzed by GC-MS of trimethylsilyl derivatives as previously described (*22*, *28*). For some strains, including the small number of strains that produced no detectable trichothecenes in infected wheat heads, trichothecene production was also analyzed in cultures grown for 2−4 weeks on autoclaved rice as previously described (*22*, *28*). Each compound was identified by comparing its retention time and mass spectrum with an authentic standard.

**Statistical Analyses.** Associations between host of origin, SCAR group, and trichothecene production were analyzed using the nonparametric GEMOD procedure in SAS (*31*). This test uses $\chi$-square values to test whether associations are random. Virulence data were analyzed using the general linear model procedure of SAS.

## RESULTS

**Genetic Variability.** The incidence of recovery of *F. graminearum* was high for all plant samples and all farms tested in 1993, 1997, and 2000. *F. graminearum* was recovered from maize samples from all nine farms tested, wheat samples from 14 of 15 farms tested, rice samples from 11 of 13 farms tested, weed samples from all four farms tested, and soil debris samples from three of four farms tested. Data for the three sample years and for individual farms were combined for statistical analysis and presentation. Five hundred seventy-six strains were identified as *F. graminearum* by morphology and by formation of species specific, sequenced, characterized PCR products, previously designated SCARs 1, 2, 3, 4, and 5 (*21*, *22*).

One hundred forty strains of SCARs 1, 2, 3, and 5 were analyzed further by a preliminary AFLP analysis in which each strain was scored for the presence or absence of 64 bands. Fifteen invariant loci were removed from the analysis because they did not differentiate among the strains and only served to increase the overall genetic similarity among strains. AFLP profiles of the 49 polymorphic loci indicated that the SCAR markers actually divide the Nepal population into three distinct lineages corresponding to SCAR1, SCAR2, and a third lineage comprising SCAR3 and SCAR5 (hereafter SCAR3/5). For 10% of the strains, the SCAR marker incorrectly identified the lineage, and when this occurred, the strain was moved to the correct group based on the AFLP profile.

On the basis of the polymorphic SCAR marker, the *F. graminearum* population of 576 strains from Nepal was genetically variable. For all plant hosts, 8% of the strains were SCAR1, 33% were SCAR2, 58% were SCAR3/5, and 0.5% were SCAR4 (**Table 1**). Because of their very low frequency, SCAR4 strains were not studied further. Each plant host yielded strains with SCAR1, SCAR2, and SCAR3/5 markers, but SCAR lineages were not isolated randomly across hosts ($\chi^2 = 55.445$, $p < 0.0001$, df = 8) (**Table 2**). In particular, the frequency of SCAR2 and SCAR3/5 varied according to host. Strains recovered from maize had a higher than expected frequency of SCAR2 (52%) as compared to the overall frequency of SCAR2 and a lower than expected frequency of SCAR3/5 (44%). Strains recovered from wheat displayed the opposite pattern, with SCAR3/5 at a frequency of 85% and SCAR2 at a frequency of only 12%. Specific contrasts of the frequency distributions of SCAR markers from each host indicated that the distribution on wheat differed from the distributions found on all other hosts (**Table 2**). The frequency distribution of SCAR markers among strains from maize differed from the distribution found on rice and weeds, in addition to the significant difference with wheat. Furthermore, the frequency distributions of SCAR markers among strains from soil debris and weeds were different, although debris and weeds were sampled at the same time from the same fields.

**Table 2.** Likelihood Ratio Contrasts Used to Compare the Frequency Distributions of SCAR Markers among *F. graminearum* Strains from Each Host

| contrast | $X^2$ | probability that distributions are similar |
|---|---|---|
| wheat vs debris | 27.63 | <0.0001 |
| wheat vs maize | 32.57 | <0.0001 |
| wheat vs rice | 12.57 | 0.0004 |
| wheat vs weeds | 7.94 | 0.0048 |
| maize vs debris | 0.52 | 0.4691 |
| maize vs rice | 5.90 | 0.0151 |
| maize vs weeds | 4.12 | 0.0423 |
| debris vs rice | 3.33 | 0.0682 |
| debris vs weeds | 2.43 | 0.1193 |
| rice vs weeds | 0.03 | 0.8645 |

**Trichothecene Production.** Of the 261 strains of *F. graminearum* tested for trichothecene chemotype, 30% produced DON, 67% produced NIV, and 3% produced no detectable trichothecenes in infected wheat heads or in culture (**Table 1**). In wheat heads and in laboratory cultures, DON producers accumulated DON as the major trichothecene, as previously reported (*31*). 15-Acetyldeoxynivalenol was usually observed as a minor cometabolite, and small amounts of 3-acetyldeoxynivalenol were occasionally detected. In wheat heads, NIV producers accumulated NIV as the major trichothecene, but in laboratory cultures, both NIV and 4-acetylnivalenol were produced. DON was occasionally produced by NIV producers but was usually present at less than 1% of the level of NIV or 4-acetylnivalenol.

In sharp contrast to the distributions of SCAR markers, the ratio of DON producers to NIV producers was similar for *F. graminearum* populations isolated from each host ($\chi^2 = 8.342$, $p = 0.4008$, df = 8) (**Table 1**), suggesting that the trichothecene chemotype by itself does not affect the host range of a strain. The trichothecene chemotype, however, differed among SCAR groups ($\chi^2 = 70.63$, $p < 0.0001$, df = 4) (**Table 3**). Among SCAR1 strains, 95% produced DON and only 5% produced NIV, while among SCAR2 strains, 94% produced NIV and only 1% produced DON. SCAR3/5 contained a mixture of 35% DON producers and 63% NIV producers.

The possibility of genetic linkage between the trichothecene biosynthetic gene cluster and the SCAR markers was studied by locating both loci on the AFLP-based genetic linkage map of *F. graminearum*. The locus governing production of DON or NIV was previously mapped to the trichothecene gene cluster on linkage group I (*28*). One parent of this cross carried the

**Table 3.** Frequencies of SCAR Markers and Trichothecene Chemotypes among *F. graminearum* Strains

| SCAR marker[a] | number of strains with each chemotype | | | |
|---|---|---|---|---|
| | DON | NIV | none | total |
| 1 | 19 | 1 | 0 | 20 |
| 2 | 1 | 69 | 3 | 73 |
| 3/5 | 56 | 102 | 4 | 162 |
| all markers | 76 | 172 | 7 | 255 |

[a] Markers, chemotypes, and abbreviations as in **Table 1**.

SCAR1 marker and produced DON, whereas the other parent carried the SCAR5 marker and produced NIV. Analysis of polymorphic SCAR1 and SCAR5 markers among 96 progeny of the mapping cross found that all progeny contained only one of the two parental SCAR markers, indicating that SCAR1 and SCAR5 were allelic. In addition, 56 progeny were recovered that were either SCAR1 NIV producers or SCAR5 DON producers. Recovery of recombinant progeny allowed mapping of the SCAR locus to linkage group 5. Subsequently, the SCAR locus was located on contig 1.72 and the trichothecene core cluster was located on contig 1.159 of the *F. graminearum* genome sequence (*F. graminearum* Database, Broad/Whitehead Institute/ Massachusetts Institute of Technology Center for Genome Research [http://www.genome.wi.mit.edu], last accessed 28 June 2004).

**Virulence on Wheat.** The virulence of 246 strains of *F. graminearum* was assessed by measuring wheat head blight (AUDPC) and seed yield (mg) per head after fungal inoculation. Ideally, the virulence of Nepalese strains would be assessed using a wheat landrace that is commonly grown in Nepal. A test against a wheat landrace would allow us to assess directly the degree to which a strain has adapted evolutionarily to infect wheat. However, we did not possess enough seed from the landrace to test adequately the virulence of all of the strains. Therefore, we chose to test the Nepalese strains against a standard U.S. cultivar, Wheaton. Virulence tests against Wheaton were validated by inoculating a group of 35 strains, which collectively encompassed each plant host, chemotype, and SCAR group, onto a Nepalese landrace of wheat. Although average virulence on the landrace was lower than that measured on Wheaton, the relative ranking of strain virulence did not change significantly (data not shown).

The virulence on cultivar Wheaton differed among *F. graminearum* strains and was influenced by both trichothecene chemotype and SCAR grouping. DON producers had higher mean AUDPC values than NIV producers ($F = 374.39$, $p < 0.0001$) (**Table 4**). As a result of the higher disease severity, DON producers also caused significantly lower yields ($F = 156.72$, $p < 0.0001$). DON producers and NIV producers from Nepal had mean AUDPC values that were significantly lower than the U.S. standard strain GZ3639. Strain GZ3639 also caused significantly lower yields than NIV producers but caused yields similar to DON producers from Nepal.

Mean AUDPC values also differed among the three SCAR groups, with SCAR3/5 having the highest mean AUDPC and SCAR2 the lowest (**Table 4**). As expected, higher mean AUDPC values were associated with lower mean yields per head. The AUDPC for strain GZ3639 was significantly higher than all of the SCAR groups, and the yield was significantly lower. For control treatments, the AUDPC values were significantly lower than for all SCAR groups and trichothecene groups, and the yields were higher.

Unfortunately, it was not possible to determine the interaction between trichothecene and SCAR group on the full data set because SCAR1 contained only one NIV producer and SCAR2 contained only one DON producer (**Table 3**). However, it was possible to demonstrate that the effects of trichothecene chemotype and SCAR group are distinct. Within the SCAR3/5 genetic background, DON producers were on average more virulent than NIV producers ($F = 224.31$, $p < 0.0001$) (**Table 4**). Among NIV producers, those from SCAR3/5 were on average more virulent than SCAR2 strains ($F = 35.74$, $p < 0.0001$). Furthermore, DON producers from the SCAR3/5 group were more virulent than DON producers from SCAR1 ($F = 13.00$, $p < 0.001$).

**Table 4.** Mean Virulence, Measured as AUDPC, and Mean Yield of Wheat Heads Inoculated with *F. graminearum* Strains from Different Chemotypes and SCAR Groups

| group | number of strains tested[a] | AUDPC[b] | yield per head (mg)[b] |
|---|---|---|---|
| control | | 126 ± 15 | 1372 ± 53 |
| U.S. strain GZ3639 | | 1023 ± 39 | 315 ± 24 |
| DON | 74 | 873 ± 15[c] | 443 ± 14 |
| NIV | 172 | 553 ± 9 | 714 ± 13 |
| SCAR1 | 20 | 651 ± 26 | 580 ± 32 |
| SCAR2 | 70 | 364 ± 11 | 934 ± 21 |
| SCAR3/5 | 156 | 778 ± 10 | 503 ± 10 |
| SCAR3/5, DON | 54 | 964 ± 16 | 386 ± 15 |
| SCAR3/5, NIV | 102 | 679 ± 11 | 565 ± 14 |
| NIV, SCAR2 | 69 | 366 ± 11 | 934 ± 22 |
| NIV, SCAR3/5 | 102 | 679 ± 11 | 565 ± 14 |
| DON, SCAR1 | 19 | 650 ± 27 | 581 ± 33 |
| DON, SCAR3/5 | 54 | 964 ± 16 | 386 ± 15 |

[a] Eight replicate tests of control and U.S. strain GZ3639 were conducted. All other strains were tested once. Ten heads of wheat were inoculated in each test. [b] Means and standard errors of wheat head blight tests conducted in the greenhouse as previously described (*29*). [c] Means within each specified comparison differ significantly from each other. Control AUDPC and yield values differ significantly from all other means. U.S. strain GZ3639 means differ from all other means, except the mean values for SCAR 3/5 DON producers.

## DISCUSSION

DNA sequencing and other genetic analyses have identified a high level of variability among morphologically similar strains of *F. graminearum* worldwide. To date, eight lineages (*20*, *33*) as well as other genetically distinct groups of strains (*9*, *21*, *22*) have been defined. Although a large number of strains have been assessed for genetic diversity, few of them have been rigorously assessed for trichothecene diversity, especially for regions outside North America and Europe. The geographically widespread lineage 7, which predominates on wheat and maize in North America and in Europe, produces primarily DON (*20*, *24*). Lineage 6, which predominates on wheat and barley in eastern China and in Korea, produces DON and NIV (*8*, *9*, *20*, *23*).

For previous studies of genetic variability and/or trichothecene chemotype of *F. graminearum* from Nepal, approximately 70 strains were isolated from maize, wheat, and rice grain samples collected in 1993 and 1997 at several locations throughout the country. The analysis of SCAR markers and RAPD markers placed 62 strains collected in 1993 and 1997 into two groups, designated RAPD group A and RAPD group B (*21*, *22*). The analysis of trichothecene production indicated that RAPD group A contained both DON producers and NIV producers, whereas RAPD group B contained only NIV producers. There was preliminary evidence that three RAPD group A DON producers comprised a distinct subgroup, but the number of strains tested was too small to draw a conclusion. The 62 strains from Nepal also were genetically distinct from DON producers from the United States and from DON producers and NIV producers from Europe.

For the present study, 576 strains of *F. graminearum* were collected from one geographic location in the Lamjung district of Nepal. This large population included approximately 40 strains that were included in earlier studies (*9*, *21*, *22*), plus more than 500 new strains that were isolated from samples collected in 1993, 1997, and 2000. The analysis of SCAR markers, AFLP markers, and trichothecene chemotypes in the larger population of strains in the present study supports the division of *F. graminearum* from Nepal into three groups that each differ in both genotype and chemotype. The largest group of 334 strains is SCAR3/5 (RAPD group A) (*21*), which contains 34% DON producers and 63% NIV producers. The second largest group of 192 strains is SCAR2 (RAPD group B), which contains 94% NIV producers. The smallest group of 47 strains is SCAR1, previously designated a possible subgroup of RAPD group A, which contains 95% DON producers. On the basis of sequencing of *TRI101*, a trichothecene biosynthetic gene located outside the major trichothecene gene cluster, SCAR3/5 (RAPD group A), is congruent with lineage 6; SCAR2 (RAPD group B) is congruent with lineage 2, and SCAR1 is different from the eight previously defined lineages (*9*, *20*).

In the present study, the localization of the SCAR marker on the *F. graminearum* genome was completed by two methods: by utilizing a mapping cross between a DON producer and a NIV producer and by identifying the sequence in the *F. graminearum* genome database. The SCAR marker does not map to a location near known trichothecene biosynthetic or regulatory genes (*28*, *34*). On the physical map, the SCAR marker is located within an exon of a zinc finger ($C_2H_2$ type) protein. Zinc finger proteins are usually involved with gene regulation, but it is unknown, at present, what gene(s) may be regulated by this protein.

Sequence analysis of trichothecene genes in all reported lineages of *F. graminearum* (*9*, *20*, *33*) indicates that DON biosynthesis was derived from the NIV biosynthetic pathway by nonfunctionalization of *TRI13*, which encodes a cytochrome P450 monoxygenase that is required for oxygenation at C-4 (*4*, *5*). In addition, sequence analysis of DON-producing and NIV-producing strains of *Fusarium culmorum* has found that *TRI7* and *TRI13* haplotypes are conserved between *F. graminearum* and *F. culmorum* (*9*). However, distribution of DON-producing strains of *F. graminearum* on wheat worldwide suggests that DON production may have some selective advantage, and several lines of evidence support this hypothesis. First, studies with trichothecene nonproducing mutants indicate that trichothecenes increase the ability of *F. graminearum* strain GZ3639 to cause wheat head blight (*35*). Second, phytotoxicity assays indicate that DON is at least 7-fold more active than NIV in inhibiting the growth of wheat seedlings and elongation of wheat coleoptiles (*36*, *37*). Third, efforts to map quantitative trait loci associated with the pathogenicity and aggressiveness of *F. graminearum* on wheat have found that DON-producing progenies are approximately twice as virulent as NIV-producing progenies (*38*).

Associations between strain genotype, trichothecene chemotype, and virulence of *F. graminearum* from Nepal were investigated first in seedling blight assays (*22*). The mean virulence on wheat and maize seedlings did not differ between 16 strains of RAPD group A and six strains of RAPD group B. Within RAPD group A, NIV producers were as virulent as DON producers on wheat seedlings but more virulent than DON producers on maize seedlings. In a preliminary analysis of virulence on wheat heads, RAPD group B was less virulent than RAPD group A, but within RAPD group A, DON producers and NIV producers were equally virulent (*32*). That study, however, was not conclusive because only 36 strains were tested and virulence was assessed only by measuring visible head blight symptoms at one time point, 17−18 days after fungal inoculation. Both of these limitations were overcome in the present analysis, in which 246 strains were tested and statistical analysis of virulence included AUDPC and yield for each replicate wheat head of each treatment. The present study establishes that DON producers are more virulent than NIV producers across all three SCAR groups and within the SCAR3/5 (RAPD group A, lineage 6) genetic background. The relative importance of DON and NIV as virulence factors in other populations of *F. graminearum* remains to be established. Experimental approaches include genetic crosses between suitable strains within each population and construction of DON-producing and NIV-producing isogenic lines by transformation of *F. graminearum* to delete or add *TRI13*, which encodes the trichothecene C-4 hydroxylase (*4*, *5*).

Although a DON-producing population of *F. graminearum* is already well-established in North America, the potential impact of introduction of strains from other *F. graminearum* populations should be assessed. In particular, the relative frequency of DON and NIV contamination is of concern because NIV has proven more toxic than DON in most animal systems studied to date (*39*−*41*). The present study provides an example of the kind of information that is needed for risk assessment of trichothecene chemotype, virulence, and other traits that could affect the survival of introduced *F. graminearum* and its impact on agricultural systems in North America and elsewhere.

## ABBREVIATIONS USED

AFLP, amplified fragment-length polymorphism; AUDPC, area under disease progress curve; PCR, polymerase chain reaction; RAPD, randomly amplified polymorphic DNA; SCAR, sequence characterized amplified region.

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