

Genomic Differences, Mycotoxin Profiles, and Cross-Infectivity Patterns Reveal Host Adaptability of *Fusarium graminearum* Across Barley, Potato, and Soybean

Abbeah Mae Navasca

abbeah.navasca@ndsu.edu

North Dakota State University

Viviana Rivera-Varas

North Dakota State University

Harry Navasca

North Dakota State University <https://orcid.org/0009-0007-2923-8580>

Megan Orr

North Dakota State University

Upinder Gill

Zhao Jin

North Dakota State University

Christopher Toomajian

Kansas State University <https://orcid.org/0000-0002-6763-0678>

Gary Secor

North Dakota State University

Thomas Baldwin

North Dakota State University

Article

Keywords:

Posted Date: April 9th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9053932/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: There is **NO** Competing Interest.

Genomic Differences, Mycotoxin Profiles, and Cross-Infectivity Patterns Reveal Host Adaptability of *Fusarium graminearum* Across Barley, Potato, and Soybean

Abbeah Mae R. Navasca^{1*}, Viviana Rivera-Varas¹, Harry P. Navasca¹, Megan Orr², Upinder Gill¹, Zhao Jin³, Christopher Toomajian⁴, Gary Secor¹, Thomas Baldwin^{1*}

¹Department of Plant Pathology, North Dakota State University

²Department of Statistics, North Dakota State University

³Department of Plant Sciences, North Dakota State University

⁴Department of Plant Pathology, Kansas State University

*Corresponding authors

Abstract

Fusarium graminearum, the causal agent of Fusarium head blight, contaminates small-grain cereals with deoxynivalenol (DON), threatening food and feed safety. Although widely used to manage the disease, crop rotation is limited by the persistence of *F. graminearum* in infected crop residues, serving as inoculum for subsequent crops. Here, we generated high-quality genome assemblies for seven *F. graminearum* isolates from barley, potato, soybean, and winter rye and demonstrated cross-infection across hosts. All isolates shared a large core genome, but accessory gene comparison, genomewide SNP analysis, and mycotoxin profile divided them into two groups: the barley/potato (By/Po), which belong to the NA2 population and are 3-ADON producers, and the soybean/winter rye (Sy/Wr), which belong to NA1 and are 15-ADON producers. Group classification was supported by cross-infectivity data, where By/Po exhibited greater aggressiveness than the Sy/Wr group, particularly for potato inoculations. The correspondence between how the isolates are divided when using source crop, chemotype, aggressiveness, SNP, or accessory gene data suggests that various genetic factors may influence the adaptability of *F. graminearum*. Our study is the first to demonstrate the connection between cross-infectivity patterns, mycotoxin profile, and genomic variation in *Fusarium graminearum*.

Introduction

Fusarium graminearum primarily causes the devastating disease Fusarium Head Blight in North America, causing damage and economic loss to small grains, including barley (Goswami and Kistler et al., 2004; McMullen et al., 2012). The fungus survives the winter on infected crop residues such as barley straw and corn stalks. On these residues, macroconidia develop and are spread to plants and debris by rain splash or wind. Under suitable conditions, the sexual phase occurs on the contaminated plant material, producing perithecia that release ascospores forcefully into the air. These ascospores and macroconidia land on and infect vulnerable barley heads. Infected heads initially show premature bleaching, followed by dark discoloration in later stages (Schmale and Bergstrom, 2004). Harvested kernels infected with *F. graminearum* appear as shriveled ‘tombstones’ with pinkish discoloration and are often accompanied by black perithecia when the fungus is widespread (McMullen et al., 1997). Apart from kernel damage, the most

significant threat to humans and animals is the production of trichothecene toxins, particularly deoxynivalenol, which can contaminate barley feed, food, and malt used in beer production when levels exceed thresholds (McMullen et al., 1997; Schwarz et al., 2006). DON-contaminated kernels might be sold at reduced prices, downgraded, rejected, or discarded (Schwarz et al., 2006; Wilson et al., 2018).

To manage *Fusarium* head blight, one standard recommendation is a cereal-broadleaf rotation to limit pathogens from the first crop from damaging the next crop. For example, farmers grow and harvest barley, but after harvest, crop residues are left in the field. A broadleaf crop, such as soybeans or potatoes, is planted on the same field when the next season comes. Although primarily classified as a cereal fungal pathogen, *F. graminearum* poses a threat to these rotational crops, as it persists, thrives, and multiplies in crop residues, potentially serving as inoculum for the following crop. The fungus survives as a saprophyte by decomposing organic material and overwintering on crop residues, leveraging its ability to produce enzymes that break down these residues for nutrients (Fernandez, 1991; Leplat et al., 2013; Dill-Macky and Jones, 2000). However, its competitive ability decreases over time as other organisms colonize the crop residues (Leplat et al., 2013). A recent study reported <10% and 4% isolation rates of *F. graminearum* from soil samples (24 out of 300) and plant residue samples (74 out of 1,850), respectively, which is lower compared to the isolation rate of the fungus from the weed plants at 50% (406 out of 800) (Sneideris et al., 2020). Since isolation rates are low in the soil and plant residues, the authors questioned whether soil is a primary source of infection in spring compared to the plants surrounding agricultural fields. Nevertheless, *F. graminearum* has been implicated in root diseases such as dry rot in potatoes (Ali et al., 2005; Estrada et al., 2010; Tiwari et al., 2020), root rot in soybeans (Ellis et al., 2011; Cruz et al., 2020; Okello et al., 2020), and root rot in dry edible beans (Bilgi et al., 2011), as well as *Fusarium* yellowing of sugar beet (Hanson, 2006). More recently, the fungus has been reported to cause flower blight on hemp (*Cannabis sativa*) (Yulfo-Soto et al., 2022), wilting in Chinese ginseng (Cai et al., 2023), fruit rot on Ponkan (Wang et al., 2023), and tuber rot in *Gastrodia elata* (Son et al., 2025). Some studies have also examined the cross-pathogenicity of *F. graminearum*. For instance, Moparathi et al. (2021) reported an isolate of *F. graminearum* from lentil seeds that was highly aggressive on barley and wheat, but less aggressive on chickpea and lentil, and weakly aggressive on dry pea plants. Another study shows that *F. graminearum*, along with several *Fusarium* species from either soybean or corn, is pathogenic to both crops (Okello and Mathew, 2019). In a study of row crops, five of the eight most virulent strains tested for cross-pathogenicity in corn, wheat, and soybeans were *F. graminearum*, making the fungus the greatest risk of root rot in these crops. (Parikh et al., 2018). These reports demonstrate the capacity of the fungus to infect various crops but do not include the genomic data needed to investigate the regions and features that enable *F. graminearum* to have a broad host range.

Genomic studies have significantly advanced our understanding of fungal biology, particularly in the areas of adaptation and host range expansion. Certain *Fusarium* species have partitioned genomes with core and accessory regions, while others have expanded genomes characterized by large duplications. For example, *F. oxysporum* can acquire accessory regions or chromosomes from pathogenic strains, enabling it to infect crops that it previously could not attack (Ma et al., 2014). In *F. solani*, Navasca et al. (2025) described the dispensable genomes and segmental

89 duplications as drivers of genome plasticity in a pangenome study. However, in some fungal
90 species, such as *F. graminearum*, a different type of genome compartmentalization has been
91 observed (Dong et al., 2015; Talas and McDonald, 2015; Wang et al., 2017; Laurent et al., 2018;
92 Frantzeskakis et al., 2019). This compartmentalization, often referred to as a two-speed genome,
93 indicates the presence of both fast- and slow-speed subgenomes, with the fast subgenome
94 undergoing rapid evolution to facilitate infection. Several studies have described the two-speed
95 genomes of *F. graminearum* at different capacities. Using publicly available expression data,
96 Sperschneider et al. (2015) identified 29 *F. graminearum* genes that are rapidly evolving, encode
97 secreted proteins, and strongly suggest effector functions. Wang et al. (2017) also found that the
98 fast-speed subgenome is crucial for adaptation and infection, as it has a higher concentration of
99 secreted proteins, carbohydrate-active enzymes, genes involved in pathogen-host interactions,
100 pathogen-associated genes, and secondary metabolites compared to the slow-speed subgenome.
101 They further identified eight genes responsible for *Fusarium*-wheat interaction. Both studies
102 examined the genomic features of *F. graminearum* in relation to adaptation and pathogenicity, and
103 identified candidate genes linked to infection. However, none of these studies assessed the ability
104 of *F. graminearum* as a pathogen across different crops in rotation.

106 In this study, we bridged the cross-infective ability and genomic features of seven *F. graminearum*
107 isolates originating from different host crops. We sequenced these isolates and assessed variations
108 in their genomic composition and aggressiveness toward barley, potato, and soybean. We found
109 that a set of genetically similar isolates are also more aggressive in the tested crops, particularly
110 potatoes. Integrating genomic and phenotypic data identified candidate gene groups consistently
111 associated with the most aggressive isolates across all three hosts.

114 **Materials and Methods**

116 Source, Identification, and Maintenance of *Fusarium graminearum*.

117 *Fusarium* isolates were obtained from a previous collection and also from infected barley
118 and winter rye spikes. Isolates recovered from previous collections were grown on PDA plates.
119 Isolation of *Fusarium* from barley and winter rye spikes was done by dipping sections of plant
120 tissues into 70% ethanol for 1 min, followed by three washes of sterile water, and dried in sterile
121 filter paper before being placed in PDA. All isolates were purified using the hyphal tip method
122 for two rounds and were initially screened based on growth characteristics on PDA media and
123 spore morphology using a Rebel microscope (Echo, San Diego, CA). After initial screening and
124 microscopy, 30 representative isolates were considered for molecular identification. Fungal DNA
125 was extracted using a simple modified CTAB method. In brief, mycelia from 5-7 day old PDA
126 plates were homogenized in a Qiagen disruption tube (Cat. No. 69204) with 500 µl CTAB Buffer
127 using a vortex until mycelia is pulverized. The slurry mycelia was then transferred to a sterile 1.5
128 ml microcentrifuge tube and centrifuged. The supernatant was transferred to a new tube, added
129 with 200 µl isopropanol, vortexed, and centrifuged. The supernatant was discarded, 200 µl
130 isopropanol was added to the tube, vortexed, and centrifuged. All vortexing was done for 5
131 seconds and centrifuging for 3 minutes at 14,000 rpm. The supernatant was discarded, and the

tube was air-dried for 10 minutes under the laminar hood. A 50 µl molecular grade water was added to elute the DNA and stored at -20°C until use. To identify *Fusarium* species, we utilized RNA polymerase II second largest subunit or RPB2 gene recognized by the primer pair 5f2 (5'-GGGGWGAYCAGAAGAAGGC-3') and 7cr (5'-CCCATRGCTTGYYTTRCCCAT-3') (O'Donnell et al., 2022). Primers were ordered from Eurofins Genomics (Louisville, KY) in dry and salt-free form and were reconstituted to 10 µM concentration and stored at -20°C until use. A microliter of DNA was added to the real-time PCR reaction that contained 12.5 µl of SsoAdvanced Universal SYBR Green Supermix (2x) (Bio-Rad, Hercules, CA), 1 µl of each primer (10 µM), added with nuclease-free water (Invitrogen, Waltham, MA) to reach a final volume of 25 µl per reaction. We performed real-time PCR using CFX96 Touch Real-Time PCR Detection System (Bio-Rad, Hercules, CA) to amplify the *RPB2* gene in the following profile: 95 °C for 3 mins, 40 cycles of 95 °C for 1 min, 55 °C for 1 min, and 72 °C for 2 mins, with Melt Curve Analysis at 65.0 °C to 95 °C with 0.5 °C increment. The melting curve and temperature of 86.5°C were checked for each sample after each run to verify the amplification of the target gene using Bio-Rad CFX Maestro Software v.2.3 (Bio-Rad, Hercules, CA). DNA quality and concentration were measured using Biotek Cytation 5 (Agilent, Santa Clara, CA) and Qubit 2.0 Fluorometer (ThermoFisher Scientific, Waltham, MA), respectively, before submitting them to Plasmidsaurus (Eugene, OR) for amplicon sequencing. Sequence results were run in *FUSARIUM-ID* (<http://isolate.fusariumdb.org/blast.php>) (Torres-Cruz et al., 2022) to verify the target gene. The confirmed *F. graminearum* isolates were maintained in ½-strength PDA discs and stored at -20°C until use.

Cross-infectivity in barley.

Barley seeds (cv. ND Genesis) were sowed in 'cone-tainers' filled with a mixture of potting media (Promix, Quakertown, PA) and slow-release complete fertilizer (ICL Osmocote, Tel Aviv, Israel). The plants grew in the greenhouse at 25 ± 3 °C and were watered as needed until they were ready for inoculation. Barley spikes were sorted, selecting only those at the late booting stage for inoculation, where ~75-80% of the head was covered by the flag leaf sheath. Dip-inoculation method was used to inoculate the barley spikes with *F. graminearum*. To prepare the inoculum, *F. graminearum* isolates were grown in mungbean agar for 14 days at room temperature. The macroconidia were harvested by placing 2 ml of sterile distilled water (SDW) into each plate and scraping it with sterile rubber scrapers. Spores were filtered using 100 µm nylon mesh sterile cell strainer (Fisher Scientific, Waltham, MA) to remove the mycelial fragments and prepared dilutions of the spore suspension. Spore suspension was standardized to 5x10⁵ spores per ml into 40 ml of 0.25% Tween 20 solution in a 50-ml conical tube. The spore suspension was mixed by shaking the tube and then placing the suspension in a 50-ml capacity reagent reservoir (Integra, Hudson, NH). Barley spikes in the same treatment were dipped into the suspension for 5 seconds. After dipping, the head was covered with a transparent plastic bag (6.5 x 5.5 x 1.0 inch) secured with twist ties. Each isolate served as a treatment. Control treatments were dipped in sterile distilled water. Treatments were replicated eight times, with

one spike representing a replicate. After inoculation, barley plants were placed back in the greenhouse in a randomized complete block design (RCBD). After 72 hours of incubation, the plastic bags were replaced with glassine bags. The severity of infection was evaluated after 21 days by counting the infected kernels of each head divided by the total number of kernels in each head. This experiment was performed twice.

Cross-infectivity in potato.

Freshly harvested potato tubers (c.v. Red Norland) were quickly rinsed under tap water to remove the soil, then thoroughly washed with distilled water. Afterward, tubers were dried by patting them with paper towels. *F. graminearum* was cultured in PDA for five days and then added sterilized wheat kernels. The isolate was allowed to grow in the kernels for seven days before using for inoculation. To inoculate the potatoes, the modified plug injury method of Estrada et al. (2010) was followed. In this technique, a 5-mm diameter cork borer was used to remove a tissue plug 10 mm deep in the stem end of the tuber. Instead of replacing this space with mycelial plugs, one *F. graminearum*-inoculated wheat kernel was inserted. Each isolate is a treatment replicated thrice with three potatoes per replicate. Control potatoes were inoculated with a sterile wheat kernel. After inoculation, the tubers were placed in a plastic box, arranged in RCBD, and covered with a layer of moist paper towel. The boxes were sealed with plastic lids and incubated for four weeks at room temperature (~20 °C). Disease severity was determined by cutting the potato tubers in a longitudinal section from the inoculation site, scanning the potatoes using a scanner (Epson Perfection V850 Pro Photo Scanner), and measuring the lesion area using SketchAndCalc app (<https://www.sketchandcalc.com/>). This experiment was conducted twice.

Cross-infectivity in soybean.

The layered inoculation method by Garma et al. (2025) with few modifications was performed to screen the aggressiveness of *F. graminearum* in soybeans. The modifications were based on our initial tests before setting up the experiment. We sterilized foil pans, each containing 70 g of yellow corn-meal mix (Pearl Milling Company, St. Joseph, MO) and 280 g of potting media (Promix, Quakertown, PA) twice at 48-hour intervals. The corn-meal-potting media mixture was then inoculated with 30 ml of water containing 500,000 *F. graminearum* spores/ml isolates. Soybean seeds were sterilized with 70% ethanol for 10 seconds and washed with sterile distilled water thrice. Seeds were dried in sterile filter paper on a sterile plate and germinated for three days before the experiment. In a plastic drinking cup, the layer consists of the following materials added in order: 40 g potting media (Promix, Quakertown, PA), 35 g of corn-meal-potting media inoculated with *F. graminearum*, 10 g potting media, two pre-germinated soybean seeds (c.v. Williams 82), and 10 g potting media to cover the seeds. No fertilizer was added to the cups. There were eight treatments representing seven *F. graminearum* isolates and a control. Each treatment has five cups serving as replicates. All cups were stored in a growth chamber at 22 ± 3 °C with 12 hr photoperiod for 21 days. After the incubation period, each seedling was rated using an ordinal rating scale. The ordinal rating scale accounts for the germination, seed

colonization, and root lesions following a 1 to 5 scale, where 1 = germination, healthy seedling with no visible signs of colonization, 2 = germination, little colonization of the root, and 1 to 19% of the root with lesions, 3 = germination, some colonization of seed, and 20 to 74% of the root with lesions, 4 = germination, complete colonization of the seed, and 75% or more of the seedling root with lesions, and 5 = no germination, complete colonization of the seed (Ellis et al. 2011). This experiment was carried out twice.

Re-isolation of *F. graminearum*.

To complete Koch's postulate, *F. graminearum* was re-isolated from representative samples from each replicate of each treatment in all experiments following the isolation procedures mentioned above. Barley kernels, portions of potato tubers, and soybean roots were plated in two PDA plates with five sample tissues per plate.

Mycotoxin Quantification.

Barley heads inoculated with *F. graminearum* isolates were submitted to the NDSU DON Testing Laboratory for Wheat and Barley for mycotoxin analysis. DON, 3ADON and 15ADON were detected by GC-MS, according to the standard laboratory procedure (Jin et al., 2024). Briefly, each ground sample (2.50 g) was extracted with an aliquot (20.00 mL) of acetonitrile/water (84/16, v/v) for 1 hr. The resulting extract was filtered through a C18/alumina (1/3, w/w) column, and an aliquot (2.00 mL) of the filtrate was dried under compressed air at 50°C. The dried samples were derivatized with 100 µL of BSA: TMCS: TMSI (3/2/3, v/v/v). An aliquot of 1.00 mL isooctane containing Mirex (0.50 µg/mL) was added to the derivatized samples as an internal standard. Derivatization was terminated by adding 1.00 mL of NaHCO₃ (3%, w/v) solution. The derivatized mycotoxins extracted from the isooctane layer were transferred to a GC vial and then analyzed on an Agilent 6890N gas chromatograph with a 5973 Mass Selective Detector (Agilent Technologies, Santa Clara, CA) under SIM mode. The separation was performed on a 35% phenyl siloxane column (30.0 m × 250 µm, 0.25 µm nominal) (Agilent HP-35, Part Number 19091G-133). For the quantification of mycotoxins derivatives and Mirex, the following ions were employed: 295.20 for DON, 377.20 for 3ADON, 392.20 for 15ADON, and 271.90 for Mirex.

Genetic Support for Chemotype Assignments

TRI (trichothecene biosynthesis) cluster gene sequences were obtained from FungiDB (<https://fungidb.org>) for *Fusarium graminearum* reference strain PH-1 (accessed October 14, 2025). The complete coding sequences for the core biosynthetic genes TRI3, TRI4, TRI5, TRI6, TRI7, TRI8, TRI10, TRI11, TRI12, TRI13, TRI14, and TRI101 were downloaded using their respective gene identifiers FGRAMPH1_01G13111, FGRAMPH1_01G13109, FGRAMPH1_01G13107, FGRAMPH1_01G25923, FGRAMPH1_01G13117, FGRAMPH1_01G13101, FGRAMPH1_01G13121, FGRAMPH1_01G13105, FGRAMPH1_01G13103, FGRAMPH1_01G13123, FGRAMPH1_01G13113, and

FGRAMPH1_01G13119. The genome FASTA files of the following *F. graminearum* genomes were formatted as nucleotide databases with BLAST+ v2.16.0 (Altschul et al., 1990): 1) the *F. graminearum* PH1 reference genome (NCBI Accession No. GCA_000240135.3, ASM24013v3), known as a 15ADON producer, 2) *F. graminearum* genomes with documented TRI haplotypes (Dhakal et al., 2024), such as KSU23473_15ADON (NCBI Accession No. GCA_037255895.1), KSU23522_3ADON (NCBI Accession No. GCA_037213105.1), and KSU23389_NX-2 (NCBI Accession No. GCA_037074415.1), and 3) the seven *F. graminearum* isolates used in this study (By354, By355, Po378, Sy357, Sy362, Sy364, Wr386). Each TRI gene query was searched against every database. For each gene×genome, the top high scoring pairs by bitscore was retained. Hit regions were retrieved directly from the BLAST databases. For each TRI gene, the coding sequences were translated into protein sequences using the standard genetic code, and protein sequences for every TRI gene from isolates and references were combined into a single dataset. Multiple sequences alignment was performed using MAFFT v7.525 (Katoh and Standley, 2013) with default parameters. The resulting alignment was inspected and visualized in Jalview v2.11.5.1 (Waterhouse et al., 2009).

High molecular weight DNA extraction and sequencing of *F. graminearum* isolates.

Three plugs of 7-day-old *F. graminearum* isolates were placed in 125 ml potato dextrose broth in a 250 ml beaker and incubated in a shaker at 110 rpm and 25 °C. After five days, mycelia were harvested mycelia using a 100 µm nylon mesh sterile cell strainer (Fisher Scientific, Waltham, MA). The harvested mycelia were freeze-dried for a day before DNA extraction. Each freeze-dried sample was grounded in liquid nitrogen using a sterile mortar and pestle, and then DNA was extracted following the protocol of Liu et al. (2022) with the Blood and Cell Culture DNA Maxi Kit (Qiagen, Aarhus, Denmark). Briefly, each ground sample was placed in 50 mL conical tubes containing pre-warmed G2 buffer and added 15 µL of RNase. The samples were vortexed and incubated for 1.5 hours, then added 75 µL Proteinase K before returning to the incubator for an additional 1.5 hours. After incubation, the sample was centrifuged at 3,200 x g for 10 mins. The supernatant was promptly transferred to a Qiagen Genomic Tip 500/G that had been previously equilibrated with 10 mL of QBT buffer by gravity flow. A 15 ml isopropanol was then added to the eluted DNA, and the tube was gently inverted 10-20 times. The tubes were then centrifuged at 20,000 ×g for 10 minutes, and the supernatant was discarded. The DNA was collected and transferred to a 1.5 mL tube, where it was allowed to dry overnight under a laminar hood. The DNA was then suspended in 50 µL of TE buffer for the sequencing procedure. DNA concentration ranged from 200 to 700 ng/µL. DNA samples of each isolate at 50 ng/µl at 30 µl were submitted to Plasmidsaurus (Eugene, OR) for custom long-read barcoding sequencing service using 10.4.1 flow cell in Promethion (Oxford Nanopore Technologies, UK) and to Novogene (Sacramento, CA) for Illumina sequencing in NovaSeq X Plus Series (PE150) (San Diego, CA).

Genome assembly and polishing.

The Oxford Nanopore long-read genomic data was de novo assembled using NECAT v0.0.1

(Chen et al., 2021) for all the seven isolates. To improve base-level accuracy, three rounds of long-read consensus polishing were performed using Racon v1.5.0 (Vaser et al., 2017). For each round, raw Nanopore reads were aligned to the current assembly using Minimap2 v2.28 (Li, 2018). The resulting files were then used in Racon v1.5.0 where the output from each polishing round was used iteratively as input for the next. Assemblies at each stage were evaluated using Inspector v1.3 (Chen et al., 2021), a long-read-aware assembly evaluator that reports consensus quality values (QV), error types, mapping rate, and coverage depth.

Short-read polishing was then performed using paired-end Illumina data. Initial quality checks were conducted using FastQC v0.11.9 (Andrews, 2010), assessing per-base quality, adapter content, and GC bias. Reads were trimmed using Trimmomatic v0.39 (Bolger et al., 2014) with parameters *LEADING:20 TRAILING:20 SLIDINGWINDOW:4:20 MINLEN:50*, and only paired reads were retained. Synchronization of paired reads was corrected using BBTools suite v38.90 (Bushnell, 2014). Post-trimming quality improvements were confirmed with a second round of FastQC v0.11.9. Two rounds of short-read-based genome polishing were performed using Pilon v1.24 (Walker et al., 2014) where trimmed Illumina reads were aligned to the Racon-polished assemblies using BWA-MEM v0.7.17 (Li and Durbin, 2009), and alignments were sorted and indexed using samtools v1.15 (Li et al., 2009; Danecek et al., 2021). Pilon v1.24 was run to correct SNPs, small indels, and local misassemblies. Final assemblies were evaluated using both Inspector v1.3 (Chen et al., 2021), to assess base-level accuracy and structural correctness from long-read alignments, and QAST v5.2.0 (Mikheenko et al., 2018), to evaluate contiguity, GC content, ambiguous bases, and overall assembly metrics.

Repeat Masking and Structural Gene Annotation

Repeat masking was performed using the Extensive de-novo TE Annotator (EDTA) v.2.2.0 (Ou et al., 2019) to identify and classify transposable elements (TEs) in the genome assemblies. EDTA integrates multiple structure- and homology-based tools including LTRharvest, LTR_finder, LTR_retriever, TIR, and RepeatModeler, enabling comprehensive de novo TE annotation and classification. The final genome assemblies were used as input for EDTA, utilizing the default settings. The coding regions of the *F. graminearum* PH1 reference genome (accessed May 3, 2025) were provided to the pipeline to purge gene sequences in the TE library followed by soft-masking of the genomes.

Structural gene annotation of *Fusarium graminearum* genomes was performed using BRAKER3 v3.0.8 in ETP mode (Stanke et al., 2006; Stanke et al., 2008; Iwata and Gotoh et al., 2012; Gotoh, 2008; Buchfink et al., 2015; Hoff et al., 2016; Kim et al., 2019; Hoff et al., 2019; Kovaka et al., 2019; Pertea and Pertea, 2020; Bruna et al., 2021; Bruna et al., 2023; Gabriel et al., 2021; Gabriel et al., 2024), which integrates RNA-seq and protein homology evidence for accurate gene prediction. Softmasked genome assemblies were used as input. RNA-seq data consisted of nine SRA libraries: SRR32049429, SRR32049430, SRR32049431 (PRJNA260097), and SRR32797430–SRR32797435 (PRJNA260194). These datasets all represent the *F. graminearum*

PH-1 reference isolate. Protein evidence was compiled into a single FASTA file by concatenating fungal proteins from OrthoDB v.11 (Kuznetsov et al., 2023; accessed June 7, 2025), the EnsemblFungi *F. graminearum* MDC_Fg13 proteome (GCA_901446245, accessed June 8, 2025), and 921 UniProt-reviewed proteins retrieved using ‘*Fusarium*’ as a prompt (accessed June 8, 2025). Following annotation, only the longest isoform per gene was used in downstream analyses.

Genome Completeness.

Benchmarking Universal Single-Copy Orthologs (BUSCO) v5.5.0 (Seppey et al., 2019; Manni et al., 2021) in protein mode was used to assess the completeness of predicted protein-coding gene sets for each isolate, based on the presence of conserved, single-copy orthologs. The fungi_odb10 lineage dataset (creation date: 2024-01-08; 758 BUSCOs) was used, which comprises orthologous groups derived from 549 fungal species. Protein length distribution of each isolate was also determined to check the annotation quality, thereby supplementing BUSCO analysis (Nevers et al., 2023).

Gene Prediction and Functional Annotation.

Phobius (Käll et al., 2004) and SignalP 6.0 for eukaryotes (Teufel et al., 2022) were combined to screen secreted proteins. Proteins predicted as signal peptides by both tools were analyzed with EffectorP v3.0 (Sperschneider & Dodds, 2022) using default parameters to predict potential fungal effector proteins. For carbohydrate-active enzyme (CAZyme) annotation, the same signal peptide-filtered proteins were submitted to the dbCAN3 web server (Zhang et al., 2023). Predictions were based on HMMER (E-value < 1e−15, coverage > 0.35), DIAMOND (E-value < 1e−10), and dbCAN-sub HMMER (E-value < 1e−15, coverage > 0.35). Only hits supported by at least two of the three methods were considered as CAZymes. Functional gene annotation was performed using the protein sequences predicted by the BRAKER3 pipeline as input for eggNOG-mapper (Huerta-Cepas et al., 2019; Cantalapiedra et al., 2021; Buchfink et al., 2021) to identify protein domains, families, and functional signatures.

Population assignment and Genome-wide SNP Analysis.

A total of 62 publicly available Illumina short-read sequences of *F. graminearum* isolates with known population assignment from Kelly and Ward (2018) and Dhakal et al., (2024) were downloaded from NCBI and used as references to identify the population classification of *F. graminearum* isolates used in this study. A list is available in Supplementary Table 1. Reads from each isolate were aligned to the chromosome-level assembly *Fusarium graminearum* PH-1 reference genome from NCBI (GCA_000240135.3, ASM24013v3, Accessed: June 26, 2025) using BWA v0.7.17 (Li and Durbin, 2009), followed by joint single-nucleotide polymorphisms (SNPs) calling. Variants were identified using bcftools v1.17 (Danecek et al., 2021) and were filtered to retain high-quality, bi-allelic SNPs (QUAL≥30, DP≥5). Identity-by-state (IBS) similarity was calculated across SNPs using SNPRelate v.1.32.2 (Zheng et al., 2012), and pairwise genetic distances were computed as 1−IBS. Genetic relationships among isolates were

inferred using the neighbor-joining algorithm, and the tree was visualized using iTOL version 6.0 (Letunic and Bork, 2024). Genomic variants among the seven *F. graminearum* isolates were also identified through joint SNP calling, followed by functional annotation using a custom SnpEff v.5.1 (Cingolani et al., 2012) database built from the *F. graminearum* PH-1 genome.

Orthogroup Classification and Gene Functional Annotation.

OrthoFinder v.2.5.5 (Emms and Kelly, 2019) identified clusters of orthologous proteins between seven *F. graminearum* strains. Protein-coding genes from each isolate were then annotated using eggNOG-mapper v2.1.13 with the eggNOG 5.0 database, employing DIAMOND v.2.0.7 (Huerta-Cepas et al., 2019; Cantalapiedra et al., 2021; Buchfink et al., 2021) for sequence alignment. Orthogroup assignments from OrthoFinder were integrated with eggNOG GO annotations to generate presence-absence matrices and identify orthogroups conserved across all isolates as well as those unique to individual isolates, host groups, or aggressiveness levels.

Gene Content Across Fast and Slow Subgenomes.

To delineate fast- and slow-evolving genomic regions, SNP density patterns were used to define genomic compartments for each of the seven *F. graminearum* isolates relative to the PH-1 reference genome. For each isolate, SNPs were identified by mapping Illumina reads to the PH-1 reference genome, and VCF files were generated independently for each isolate. SNPs from each VCF were counted in non-overlapping 25 Kb windows across each chromosome. SNP density distributions were modeled using a two-component Gaussian Mixture Model (GMM) implemented in the scikit-learn package v1.4.2 in Python. This unsupervised approach partitioned genomic windows into high- and low-SNP density clusters. The cluster with the higher mean SNP density was labeled "fast-evolving," while the cluster with the lower mean was designated as "slow-evolving". To further refine the assignments and reduce noise at window boundaries, a Hidden Markov Model (HMM) in the hmmlearn package v0.3.0 was implemented. The HMM used two hidden states corresponding to fast and slow subgenomes and assigned each 25 kb window based on both SNP density and transition probabilities between adjacent windows. The final classification for each window was derived from the HMM outputs. Following compartment assignment, genomic features, including secreted proteins, carbohydrate-active enzymes (CAZymes), and predicted effectors, were quantified within the fast- and slow-subgenomes for each isolate. Genomic bins not assigned to the fast or slow subgenome, such as regions present in the isolate assemblies but absent from the PH-1 reference, or vice versa, were excluded from downstream density calculations.

Statistical analyses.

Genomic data comparisons. All genomic statistical analyses were performed in R v.4.3.1. Non-parametric tests were selected due to the small sample sizes and non-normal distributions of the genomic features analyzed. Paired fast-slow subgenome differences were tested across isolates using Wilcoxon signed-rank tests, while between-group comparisons were assessed

using Wilcoxon rank-sum tests. Categorical enrichment analyses (e.g., subgenome or functional-category enrichment) were conducted using Fisher's exact tests, and comparisons involving all seven isolates were analyzed with Kruskal–Wallis tests. All figures were generated using ggplot2 v3.5.2.

Mycotoxin profiles. Mycotoxin data (DON, 3-ADON, 15-ADON, and Total DON) were analyzed using a one-way ANOVA to test for differences among isolates. Normality and homogeneity of variance were confirmed using the Shapiro–Wilk and Levene's tests, respectively, and because both assumptions were met ($P > 0.05$), ANOVA was applied. When significant isolate effects were detected, pairwise differences were evaluated using Tukey's Honest Significant Difference (HSD) test. All statistical analyses and visualizations were conducted in R using the stats v.4.2.0, car v.3.1-3, ggplot2 v.3.5.2, and ggpubr v.0.6.1 packages.

Cross-infectivity tests. All cross-infectivity tests were conducted twice. To determine whether data from the two experiments could be combined, Levene's test was used to assess homogeneity of variances. Data sets were pooled if no significant difference in variance was detected ($P > 0.05$). Each data set was then evaluated for both variance homogeneity (Levene's test) and normality (Shapiro-Wilk test). For barley, percent severity data met the assumption of normality but not homogeneity of variances; thus, Welch's ANOVA was applied, followed by Games-Howell post hoc comparisons. For potato lesion area data, the assumptions failed for both tests; therefore, the Kruskal-Wallis test was used, followed by Dunn's test with Bonferroni correction for multiple comparisons. In the discrete ordinal data rating scale for root rot in soybeans, Relative Treatment Effects (RTEs) was computed using the formula $RTE_i = [\frac{Ri_i}{N} - 0.5] / N$ where Ri_i is the mean rank of observations in treatment i , and N is the total number of observations. RTE values range from 0 to 1, where 0.5 indicates average severity, values above 0.5 indicate greater severity and values below 0.5 indicate lesser severity. To estimate the uncertainty around each RTE, we performed non-parametric bootstrapping (1000 resamples) and calculated 95% percentile confidence intervals. All statistical analyses were performed in R. Base R functions were used for ANOVA, Kruskal-Wallis, and Shapiro-Wilk Normality Tests. Additional packages included 'car' for Levene's test, 'FSA' for Dunn's test with Bonferroni correction, rstatix for Welch's ANOVA and Games-Howell test, and 'boot' for bootstrapping confidence intervals.

Results

Isolate aggressiveness varies in barley, potato, and soybean.

We identified seven isolates as *F. graminearum* by sequencing the *RPB2* gene. Two isolates are from barley, three from soybean, one from potato, and one from winter rye. Fig. 1 shows the cultural morphology, location, and source crop of the seven *F. graminearum* isolates used in this study. All cross-infectivity tests conducted across experiments for each crop exhibited comparable variances (barley: $P = 0.3171$; potato: $P = 0.2360$; soybean: $P = 0.7136$) using Levene's test, thereby allowing the data to be combined for analysis. In barley, severity levels were relatively similar among isolates, except for Sy357 and Wr386, which exhibited the highest

severity, and Sy364, which exhibited the lowest (Table 1 and Fig. 2). In potatoes, the aggressiveness of the By354 and By355 barley isolates was not significantly different from the Po378 potato isolate and the two soybean isolates, Sy362 and Sy364. The other soybean isolate, Sy357, has a similar degree of aggressiveness to the Wr386 winter rye isolate, with both exhibiting the same lesion area as Mock. In soybeans, comparisons among treatments were expressed in RTE, where 0.50 represents the average severity. Values greater than 0.5 indicate greater severity, while values lower than 0.5 denote lower severity. Among treatments, the highest RTE (0.74) was observed in By355, followed by Sy357 (0.59) and Po378 (0.59). The By354 (0.53) and Sy362 (0.56) are slightly above the 0.50 average severity, while Sy364 (0.44) and Wr386 (0.47) fall below the average severity mark. All *F. graminearum* isolates were recovered from the representative infected tissues, confirming Koch's postulate.

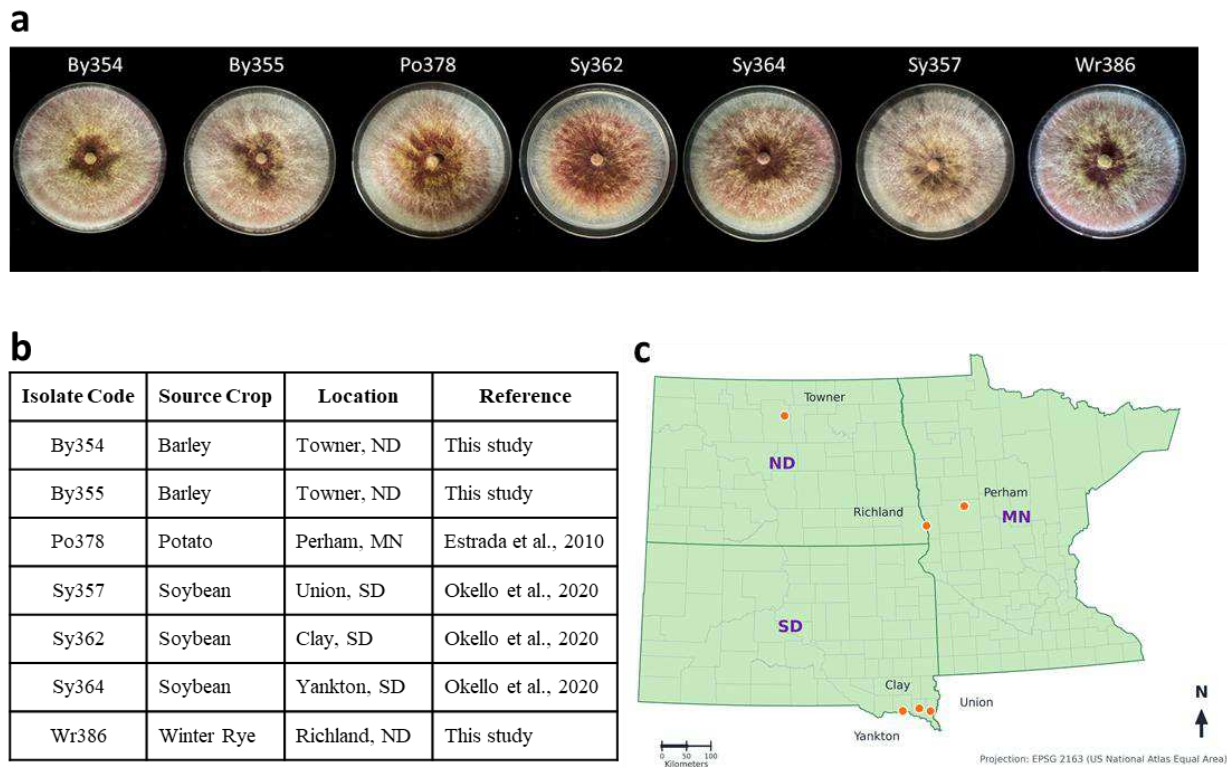


Figure 1. The seven *Fusarium graminearum* isolates used in this study. **a)** *F. graminearum* showing the typical carmine pigmentation on PDA plate at 7 days of growth in the dark at room temperature. **b)** Isolate code and source crop of *F. graminearum* isolates **c)** Map showing geographical locations of each isolate. The following codes indicate the sources of the isolates: By = barley, Sy = soybean, Po = potato, Wr = winter rye.

Overall, all isolates displayed aggressive behavior in barley, confirming that *F. graminearum* is a primary pathogen in this crop. Notably, when inoculated to barley, Sy357 isolate from soybean

has effects comparable to those of the Wr386 isolate, with both being a little bit more aggressive than the barley By354 and By355 and potato Po378 isolates, although differences were not significant. Most isolates are aggressive when inoculated into the crops from which they were initially isolated, except isolate Sy364 in soybeans, which has an RTE of only 0.44, below the average severity. These variations in aggressiveness among isolates reflect diverse pathogenic potential of *F. graminearum* within multi-host agrosystems. We ranked the isolates based on the statistical analysis of their aggressiveness per crop (Supplementary Table 1). Isolates with a sum of ranks below the median (equal to 12) were considered most broadly adapted across crops, and these are By355, followed by both By354 and Po378 (Table 1). The seven *F. graminearum* isolates used in this study are hereafter referred to as cross-infectivity isolates.

Table 1. Severity measurements for each *F. graminearum* isolate in barley, potato, and soybean.

Isolate Code	Severity in barley spikes (%) ¹	Severity in potato tubers, (mm ²) ¹	Severity in soybean Roots (RTE + 95% CI)	Sum of Rank ²
Mock	0.0 c	57.3 d	0.06 [no variation]	N/A
By354	60.5 ab	221.3 a	0.53 [0.42, 0.63]	10
By355	60.2 ab	215.3 a	0.74 [0.64, 0.82]	7.5
Po378	60.5 ab	235.5 a	0.59 [0.45, 0.72]	10
Sy357	75.2 a	64.9 cd	0.59 [0.46, 0.73]	12
Sy362	51.9 ab	98.7 abc	0.56 [0.45, 0.67]	13
Sy364	43.6 b	100.3 ab	0.44 [0.34, 0.56]	17.5
Wr386	74.4 a	87.0 bcd	0.47 [0.36, 0.59]	14
<i>P</i> -value	0.0002	0.0001	NA	

¹Values with the same letters are not significantly different based on respective statistical analysis detailed in Materials and Methods.

² Isolates with a sum of ranks below the median (equal to 12) are considered most adaptive. Ranks of each measurement are shown in the Supplementary Table 2.

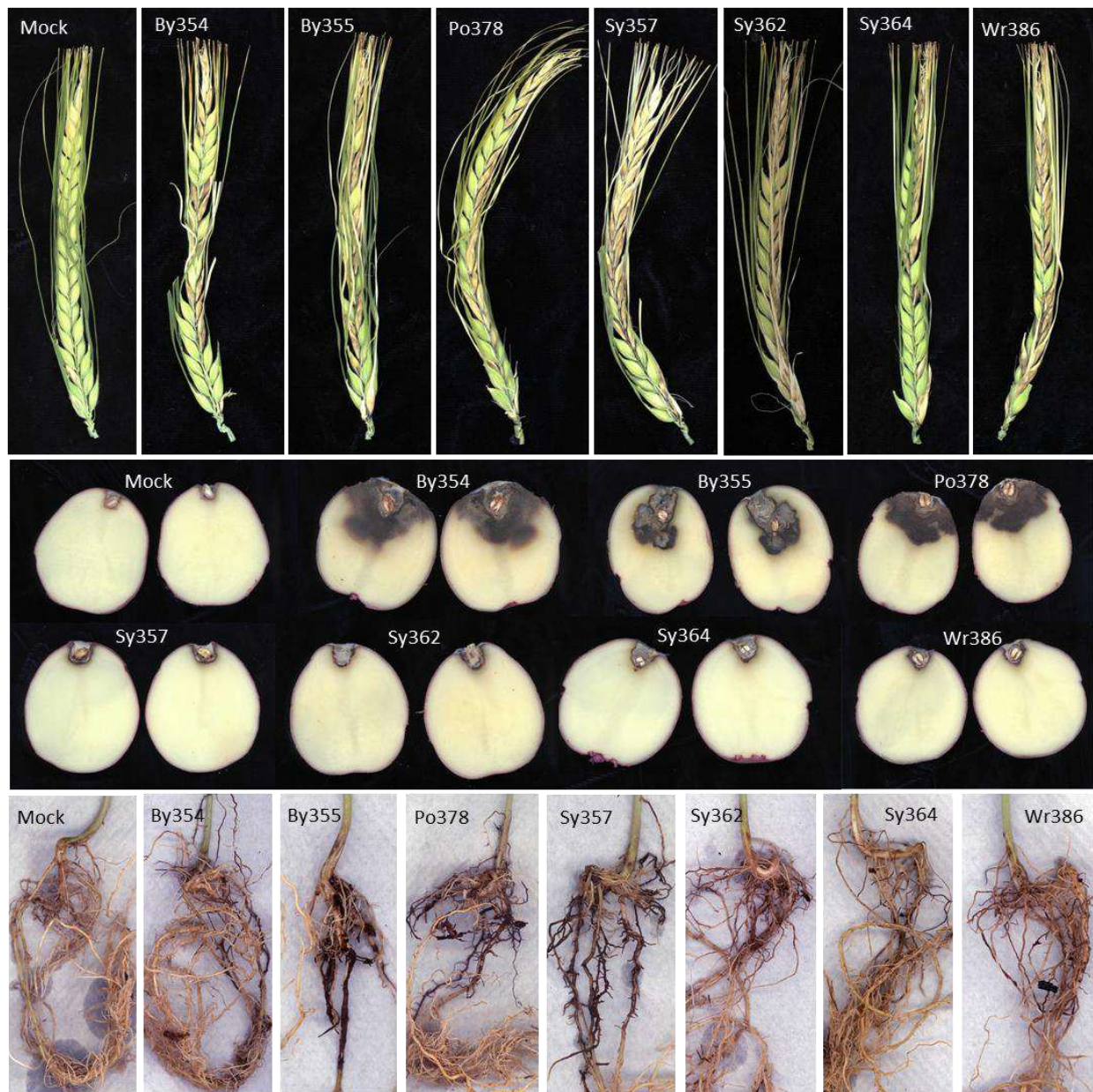


Figure 2. Representative samples of barley spikes (upper panel), potato tubers (middle panel), and soybean roots (lower panel) inoculated with *F. graminearum* isolates. The following codes indicate the sources of the isolates: By = barley, Sy = soybean, Po = potato, Wr = winter rye.

Cross-infectivity isolates are 3-ADON and 15-ADON producers.

Mycotoxin production varies strongly among cross-infectivity isolates (Fig. 3E). Overall, winter rye (Wr386) and soybean-derived isolates (Sy357, Sy362, Sy364) had lower total mycotoxin compared to barley and potato isolates (By354, By355, Po378), with Po378 being the most aggressive mycotoxin producer. DON levels mirror total mycotoxin patterns, indicating that DON is the dominant contributor to toxin across isolates. Potato and the barley isolates produce

significant 3-ADON, while soybean and winter rye isolates produce only trace amounts. 15-ADON production is concentrated in soybean isolates, particularly Sy357. Barley isolates show no 15-ADON, while the potato isolate had a small amount. It was interesting to note that the winter rye isolate Wr386 yielded 3-ADON levels similar to those of By354. However, these absolute levels were lower than the typical 3-ADON production observed across the barley and potato isolates. Total mycotoxin levels are highest in Po378, highlighting its strong toxigenic capacity. To provide genetic support for this chemotype classification based on mycotoxin analysis, TRI gene sequences were examined, with TRI8 specifically analyzed because it discriminates between 3-ADON and 15-ADON chemotypes (Alexander et al., 2011). TRI8 protein sequence alignment and phylogenetic analysis showed clear clustering of isolates according to their chemically determined chemotypes. Isolates identified as 3-ADON by mycotoxin analysis grouped with 3-ADON reference strains, while 15-ADON isolates clustered with 15-ADON references (Supplementary Fig. 1). These clustering patterns were consistent across TRI8 alignment and neighbor-joining tree (Supplementary Fig. 2), providing genetic support for chemotype classifications inferred from mycotoxin measurements.

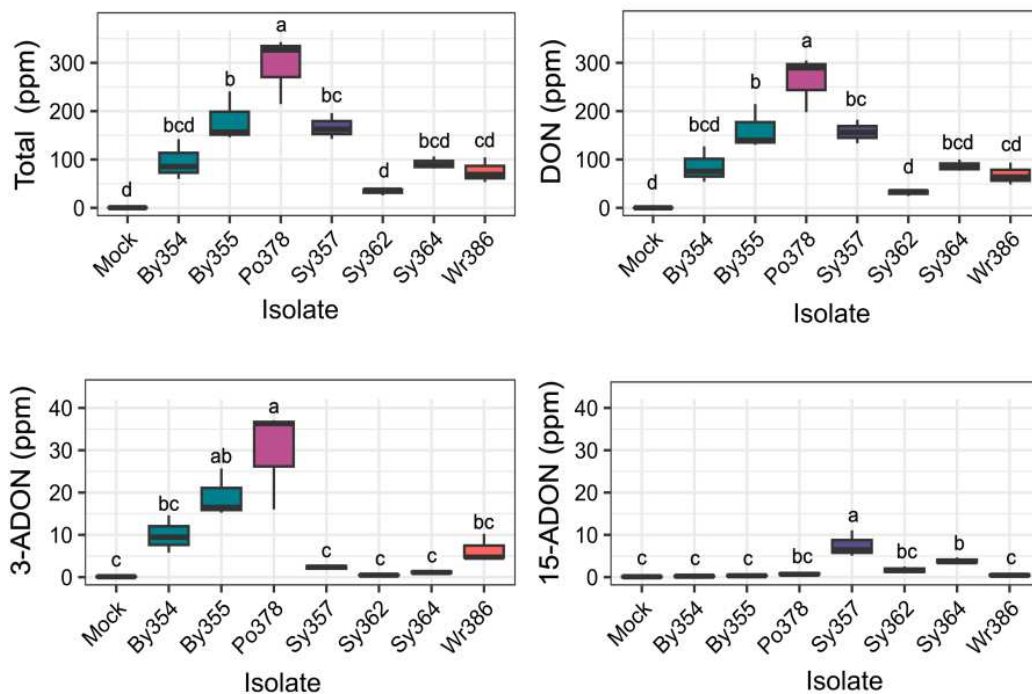


Figure 3. Mycotoxin production profiles shown as total (DON + 3-ADON + 15-ADON), DON, 3-ADON, and 15-ADON of cross-infectivity isolates inoculated on barley heads. Barley isolates (By354, By355, Po378) exhibited high DON and strong 3-ADON production with no detectable 15-ADON, consistent with 3-ADON chemotypes. Soybean isolates (Sy357, Sy362, Sy364) produced 15-ADON, confirming 15-ADON chemotypes, with Sy357 showing the highest 15-ADON output. The winter rye isolate (Wr386) produced very low levels of both ADON forms.

Although 3-ADON concentration was marginally higher than 15-ADON, the total absolute levels remained below the typical 3-ADON chemotype production range observed in the barley and potato isolates. Different letters above the bars indicate significant differences between groups, determined by ANOVA and Tukey's HSD test at a 95% level of significance. Together, these panels illustrate clear chemotype groupings and highlight isolate-specific differences in toxin production intensity.

Assembly quality improved through multiple rounds of polishing. Draft genome assemblies created with NECAT v0.0.1 (Chen et al., 2021) showed moderate consensus quality values (QV), ranging from 43.6 to 44.6 across isolates. After three rounds of long-read polishing with Racon v.1.5.0 (Vaser et al., 2017), QV values increased notably, especially in isolate By354 (from 44.4 to 47.5). Two additional rounds of short-read polishing with Pilon v1.24 (Walker et al., 2014) further raised QV scores for all isolates, reaching final values as high as 48.7. Although mapping rates consistently remained high (80–88%) at all steps, indicating stable alignment efficiency, base-level accuracy improved significantly. Small-scale assembly errors, evaluated using Inspector v1.0, decreased notably. The assemblies contained over 1,100 small-scale errors per isolate, which were reduced by more than 50% after Racon v1.5.0 and decreased to approximately 350–560 after Pilon v1.24. Structural errors, such as expansions, collapses, and switches, were also minimized, with all final assemblies containing only a single structural error per isolate. Collectively, these results demonstrate that multiple rounds of hybrid polishing significantly enhance both consensus accuracy and structural integrity of fungal genome assemblies. While polishing procedures improved overall base accuracy, we noted that telomeric repeats present in the initial long-read assembly were reduced or lost during polishing, likely due to their repetitive nature, which caused short-read mapping ambiguity. Statistics on each polishing step are shown in Supplementary Tables S3-S6.

Final assemblies exhibit high consensus accuracy and contiguity.

The final assemblies, polished through three rounds of Racon v1.5.0 (Vaser et al., 2017) and two rounds of Pilon v1.24 (Walker et al., 2014), were assessed using both Inspector v1.3 (Chen et al., 2021) and QUAST v5.2.0 (Mikheenko et al., 2018). Inspector v1.3 confirmed high consensus accuracy across all isolates, with final QV scores between 44.5 and 48.7 and minimal structural errors with only one per isolate. Small base errors were also markedly reduced to approximately 350–560, highlighting the effectiveness of short-read polishing in improving assembly accuracy (Table 2). Complementary analysis with QUAST v5.2.0 (Mikheenko et al., 2018) showed consistently high structural completeness. Each genome contained 4–5 contigs larger than 50 kbp, with total lengths ranging from about 36.5 to 37.5 Mb, consistent with the expected genome size and chromosome number for *F. graminearum*. The largest contigs reached 11.8 Mb, and N50 values surpassed 9 Mb across all isolates, indicating strong contiguity. GC content remained stable at approximately 47.8%, with zero N's per 100 kbp in all assemblies, suggesting no

567 ambiguous bases. Additional continuity metrics, such as L50 and L90, verified that only 2–4
568 contigs were needed to cover 50–90% of each genome (Table 2). These results, collectively,
569 support the high accuracy, completeness, and reliability of the final assemblies. The final
570 assembly statistics are presented in Table 2.

571

572

573

574 Table 2. Assembly and quality statistics of *F. graminearum* genomes.

575

Metric	Cross-infectivity isolates						
	By354	By355	Po378	Sy357	Sy362	Sy364	Wr386
Contig statistics							
Number of contigs	5	4	4	4	4	4	4
Total length	37,562,703	36,910,886	37,132,289	36,592,799	37,000,554	36,791,154	36,562,154
Longest contig	11,854,523	11,862,830	11,828,528	11,658,933	11,892,581	11,826,751	11,712,009
N50	9,072,070	9,048,981	9,129,084	9,084,688	9,103,329	9,077,665	9,079,124
N90	7,994,273	7,986,339	8,056,136	7,863,009	7,882,176	7,904,639	7,805,967
GC (%)	47.86	47.99	47.95	48.01	47.92	47.97	48.04
Read to contig alignment							
Mapping rate (%)	80.56	88.42	80.28	83.97	84.22	82.63	87.40
Split-read rate (%)	0.56	0.93	0.68	0.65	0.49	0.92	0.68
Depth	58.72	108.87	59.75	77.91	101.24	88.32	94.37
Structural errors							
Small-scale assembly error (per Mbp)	10.25	14.39	9.29	15.41	9.95	9.46	9.49
Total small-scale assembly error	385	531	345	564	368	348	347
Base substitution	9	117	0	227	2	0	0
Small-scale expansion	371	401	340	327	354	345	343
Small-scale collapse	5	13	5	10	12	3	4
QV Score	48.72	45.61	46.52	47.02	46.00	44.47	45.14

576

577

578 Repetitive elements vary in abundance and composition among isolates.

579 Total interspersed repeats range from 1.22% to 2.22%, with the lowest percentage observed in
580 Wr386 and the highest in By354 (Table 3). Some specific repeat classes were observed in certain
581 isolates but not in others. For instance, unclassified DNA transposons and unknown LTRs were
582 found in By354, Sy357, and Po378, but not in the other isolates. Furthermore, none of the
583 soybean isolates (Sy357, Sy362, and Sy364) and the winter rye isolate (Wr386) contained hAT
584 repeats. Most repeat classes showed similar numbers and percentages across the isolates, except
585 for Tc Mariner, where the barley and potato isolates By354, By355, and Po378 had higher counts
586 of 89 (0.25%), 72 (0.28%), and 74 (0.15%), respectively, compared to the others, which had only

1-2 counts, accounting for roughly 0.1%. Additionally, the specific repeat class Kolobok was found exclusively in By355, with 24 counts representing 0.15%.

Table 3. Counts and percentage distributions of repeat classes of *F. graminearum* genomes.

Class			Description	Cross-infectivity isolates						
				By354	By355	Po378	Sy357	Sy362	Sy364	Wr386
DNA transposons	Unclassified		Counts	25	N/A	29	18	N/A	N/A	N/A
			Masked (%)	0.14	N/A	0.16	0.03	N/A	N/A	N/A
	TIR	CACTA	Counts	36	37	32	43	34	36	35
			Masked (%)	0.22	0.23	0.2	0.24	0.21	0.23	0.23
		Kolobok	Counts	N/A	24	N/A	N/A	N/A	N/A	N/A
			Masked (%)	N/A	0.15	N/A	N/A	N/A	N/A	N/A
		Mutator	Counts	38	37	35	43	38	38	36
			Masked (%)	0.21	0.2	0.21	0.27	0.23	0.21	0.21
		PIF Harbinger	Counts	5	6	5	7	6	6	6
			Masked (%)	0.05	0.05	0.05	0.04	0.04	0.04	0.04
		Tc1 Mariner	Counts	89	72	74	2	2	2	1
			Masked (%)	0.25	0.28	0.15	0.01	0.01	0.01	0.00
		hAT	Counts	1	1	1	N/A	N/A	N/A	N/A
			Masked (%)	0.01	0.01	0.01	N/A	N/A	N/A	N/A
	Non TIR	Helitron	Counts	18	17	13	12	15	14	11
			Masked (%)	0.35	0.37	0.3	0.25	0.35	0.37	0.29
Retrotransposons	LTR	Gypsy	Counts	196	110	166	109	105	97	123
			Masked (%)	0.58	0.26	0.6	0.37	0.62	0.29	0.37
		Unknown	Counts	28	N/A	14	4	N/A	N/A	N/A

			Masked (%)	0.07	N/A	0.02	0.02	N/A	N/A	N/A
	SINE	tRNA	Counts	133	133	123	121	133	117	118
			Masked (%)	0.04	0.04	0.03	0.04	0.04	0.03	0.03
		Unknown	Counts	30	30	30	30	30	30	30
			Masked (%)	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Repeat fragment			Counts	238	271	171	93	372	243	114
			Masked (%)	0.31	0.1	0.1	0.03	0.62	0.27	0.04
Total			Counts	837	738	693	482	735	583	474
			Masked (%)	2.22	1.71	1.85	1.30	2.14	1.46	1.22

Gene model counts vary among high-quality genomes of cross-infectivity isolates.

Even with nine sets of RNA-seq data, BRAKER3 v3.0.8 in ETP mode (Stanke et al., 2006; Stanke et al., 2008; Iwata and Gotoh et al., 2012; Gotoh, 2008; Buchfink et al., 2015; Hoff et al., 2016; Kim et al., 2019; Hoff et al., 2019; Kovaka et al., 2019; Pertea and Pertea, 2020; Bruna et al., 2021; Bruna et al., 2023; Gabriel et al., 2021; Gabriel et al., 2024) predicted isoforms for a subset of genes, with counts ranging from 1,287 in By355 to 1,333 in Sy362. To standardize protein-coding gene sets for downstream analyses, we retained all single-isoform genes and selected the longest isoform for genes with multiple isoforms. All cross-infectivity isolates have more than 12,000 protein-coding genes, averaging 12,342, with the highest recorded in By354 at 12,445 and the lowest observed in both Sy357 and Wr386 at 12,272 (Table 4). Secreted proteins range from 1,233 in Sy362 to 1,260 in By354, while effectors range from 433 in Sy364 to 446 in By54, and carbohydrate-active enzymes range from 284 in Po378 to 298 in By354. Consistently, the barley isolate By354 has the highest numbers of protein-coding genes, secreted proteins, effectors, and carbohydrate-active enzymes. In contrast, soybean isolates Sy357, Sy362, and Sy364, as well as winter rye isolate Wr386, have the lowest in one or two of these groups. We investigated whether the high numbers in By354 were due to the unmapped contig, but found only three secreted proteins, one effector, and two carbohydrate-active enzymes from this contig, which could not account for the differences in numbers between this isolate and the others. All genomes are over 99.0% complete, based on 758 BUSCOs. Most of these are complete and single-copy (99.2-99.4%), with only 0.4-0.5% duplicated, zero fragmentation, and only 0.6-0.8% missing BUSCOs. The average protein length is approximately 490 amino acids across all isolates, indicating strong annotation support for the BUSCO analysis (Nevers et al., 2023).

Table 4. Summary of genomic features and completeness of *F. graminearum* genomes.

Description	Cross-infectivity isolates						
	By354	By355	Po378	Sy357	Sy362	Sy364	Wr386
Protein-coding genes	12,445	12,378	12,413	12,272	12,299	12,315	12,272
Protein length	491.17	490.78	490.75	490.93	491.36	489.3	489.87
Secreted proteins	1,260	1,249	1,252	1,243	1,233	1,245	1,247
Effectors	446	443	439	438	435	433	443
Carbohydrate-active enzymes	298	294	284	295	292	296	290
Completeness in BUSCOs (%)							
Complete	99.3	99.3	99.3	99.4	99.2	99.3	99.4
Complete and single-copy	98.8	98.8	98.8	98.9	98.8	98.8	98.9
Complete and duplicated	0.5	0.5	0.5	0.5	0.4	0.5	0.5
Fragmented	0	0	0	0	0	0	0
Missing	0.7	0.7	0.7	0.6	0.8	0.7	0.6

Biosynthetic gene cluster profiles are broadly conserved among cross-infectivity isolates.

All isolates share a biosynthetic gene cluster (BCGs) profile with a total of 47, except for By354, which has an extra koraiol terpene located in the unmapped contig, bringing its total BCGs to 48 (Table 5). These BCGs are classified as terpenes (16), non-ribosomal peptide synthase NRPS (15), Type 1 polyketide synthase PKS (7), Terpene-T1PKS or NRPS-T1PKS hybrids (8), and phosphonate (1). There are 14, 16, 8, and 9 BCGs found in chromosomes 1, 2, 3, and 4, respectively, for each isolate. There are 14 BCGs with high similarity to mangicol A, autofusarium, zearalenone, 15-acetyldeoxynivalenol, fusarielin H, orcinol/orsellinic acid, α -acorenol, fusaristatin A, clavatic acid, fusatrixin/fusapentaxin/fusaoctaxin A, koraiol, chrysogine, choline, and oxyjavanicin. Another three BCGs show medium similarity to higginsianin, 15-acetyldeoxynivalenol, and lucilactaene, while two BCGs demonstrate low similarity to gipepyrone-A and squaestati S1. Although all isolates have the same BCGs, two BCGs differ in similarity confidence. One is a terpene that has medium similarity to 15-acetyldeoxynivalenol/decalonectrin in By354, By355, and Po378, but is low in other isolates. The other is an NRPS-T1PKS hybrid that shows moderate similarity to fusatrixin, fusapentaxin, and fusaoctaxin A in Wr386 but is high in other isolates. The remaining BCGs do not show similarities with known clusters.

Table 5. Predicted biosynthetic gene clusters of the cross-infectivity isolates.

Type of biosynthetic gene clusters	<i>F. graminearum</i> chromosomes
------------------------------------	-----------------------------------

	Chr 1	Chr 2	Chr 3	Chr 4	Total ¹
Terpene	4	5	2	5	16
Non-ribosomal peptide synthase (NRPS)	5	4	4	2	15
Type I Polyketide Synthase (T1PKS)	0	4	2	1	7
Terpene/T1PKS or NRPS/T1PKS hybrids	5	2	0	1	8
Phosphonate	0	1	0	0	1
Total	14	16	8	9	47

¹Another terpene, ‘koraiol’, was predicted in the unmapped contig of By354, bringing its total number of terpene to 17 and the total number of BCGs to 48.

Genome-wide SNP analysis classifies cross-infectivity isolates into two groups.

A total of 679,653 filtered SNPs were analysed for genetic relatedness among the 69 *F. graminearum* isolates. Pairwise genetic similarity was measured using the identity-by-state (IBS) metric, with values ranging from 0 (completely dissimilar) to 1 (identical at all SNP loci). The IBS matrix was converted to genetic distances (1–IBS) and displayed as a neighbor-joining tree. By/Po isolates are grouped within the same genetic cluster as NA2. In contrast, Sy/Wr isolates are clustered in NA1 (Fig. 4A). We further examined the following not-mutually-exclusive subsets of all SNPs: those variable across all seven isolates (199,342), those with fixed differences between By/Po and Sy/Wr, i.e., all By/Po have ‘allele 1’ and all Sy/Wr have a distinct ‘allele 2’ (46,247), those variable within By/Po (58,551), and those variable within Sy/Wr (157,831). Regardless of the subset, upstream/downstream variants account for more than 50% of total variation, followed by synonymous and then missense variants (Fig. 4B). The remaining classes (intergenic, intron, UTR, splice, loss of function, and others) account for minimal amounts. We also examined the positions of these SNPs within 50 Kb windows across the *F. graminearum* PH-1 chromosomes. SNP density is not evenly distributed across PH-1 chromosomes, with discrete peaks separated by regions of low SNP density (Fig. 4C). This holds true across the different SNP subsets, though the peaks are most evident in the “all” category, since it contains the most SNPs. In contrast, only a small fraction of these variants are present in the “fixed” category. The By/Po group exhibits fewer SNPs than the Sy/Wr group, indicating closer genetic similarity between barley and potato isolates compared with the greater divergence observed between soybean and winter rye isolates.

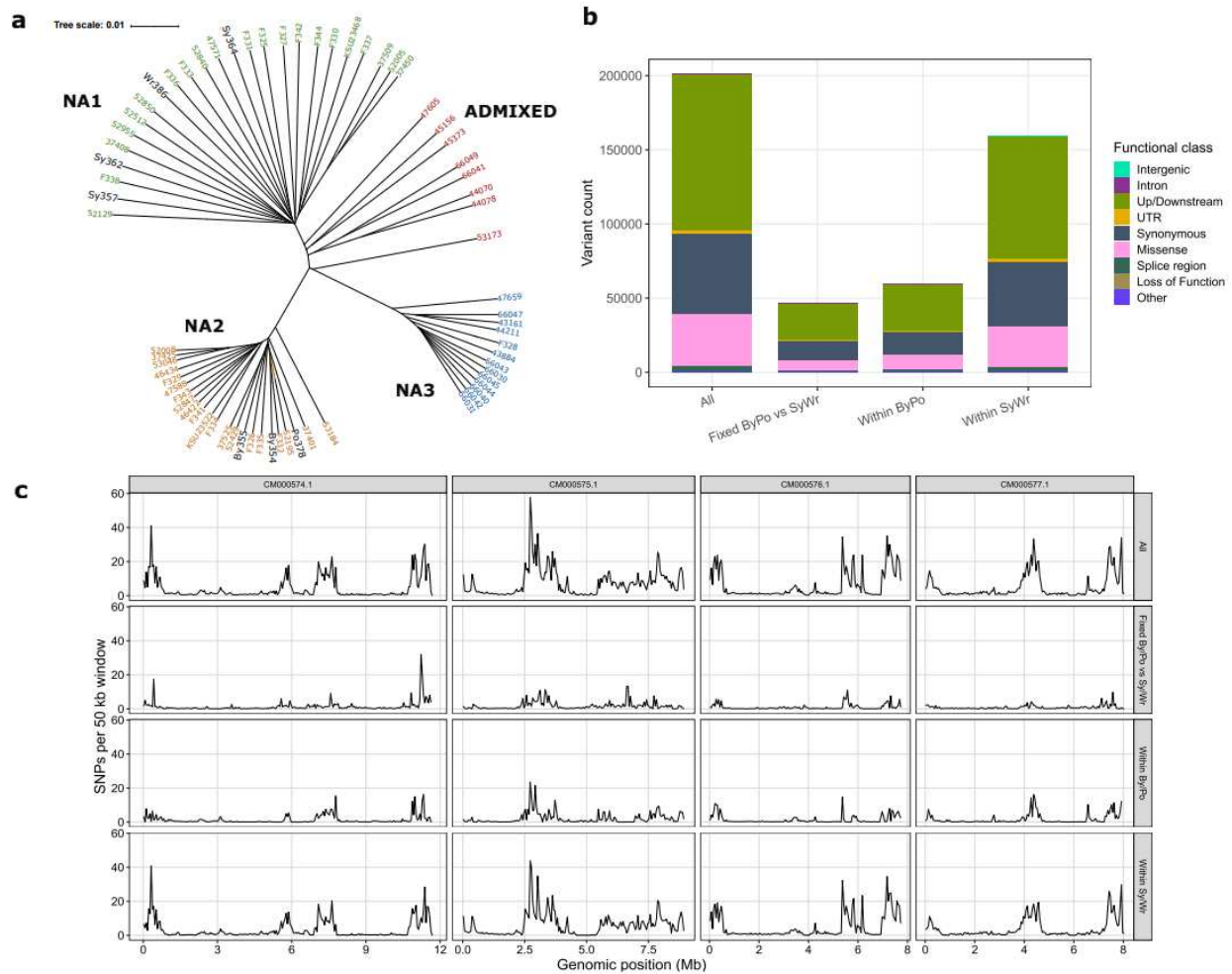


Figure 4. Population Assignment and Genome-wide SNPs Profile of *F. graminearum* Isolates. **a)** Neighbor-joining tree based on genome-wide identity-by-state (IBS) genetic distances among 69 *F. graminearum* isolates (62 reference + 7 cross-infectivity isolates). Pairwise genetic distances were calculated as $1 - \text{IBS}$ using high-quality bi-allelic SNPs. The tree shows relative genetic similarity among isolates, with branch lengths reflecting IBS-derived genetic distances. Barley and potato isolates (By354, By355, Po378) clusters within NA2 while soybean and winter rye isolates (Sy357, Sy362, Sy364, and Wr386) are grouped within NA1. **b)** Proportion of SNPs classified based on SnpEff v.5.1 annotations. Variants are included in four categories: variants segregating across all seven isolates, variants fixed between ByPo and SyWr isolates, variants segregating within ByPo, and variants segregating within SyWr. Upstream/downstream variants account for more than 50% of total variation, followed by synonymous and then missense variants while remaining classes (intergenic, intron, UTR, splice, loss of function, and others) were minimal. **c)** Genome-wide SNP density across variant categories All (all cross-infectivity isolates), ByPo vs SyWr, within ByPo, and within SyWr, showing similar genomic regions with elevated variation.

Gene Repertoire Revealed Core Gene Conservation and Two Distinct Isolate Groups

OrthoFinder analysis identified orthogroups across cross-infectivity isolates. The total number of orthogroups (OGs) per isolate was highly consistent, ranging from approximately 11,000 to 12,500 (Fig. 5A, left panel), indicating similar gene repertoire sizes across all isolates despite their genetic divergence.

The seven isolates shared a remarkably large core genome comprising 11,505 orthogroups (Fig. 5A, upper panel). This universal core accounts for the majority of gene content and reflects highly conserved functions shared across all isolates. Beyond this core, the accessory genome included orthogroups with variable presence-absence patterns. The largest number of accessory orthogroups was shared by three isolates, By354, By355, and Po378, with 91 OGs. The intersection patterns of orthogroup distribution show which specific isolate combinations share particular orthogroups (Fig. 5A, lower panel). The universal core (present in all seven isolates) dominated as the most prevalent pattern, with additional combinations revealing subset-specific orthogroups that may reflect lineage-specific adaptations.

Pairwise comparisons of orthogroup composition revealed high levels of gene content conservation across all isolates (Fig. 5B). Shared orthogroup percentages (lower red triangle) ranged from 97.6% to 99.2%, with clear clustering of isolates into two groups: a By/Po cluster (By354, By355, Po378) showing 98.8-99.2% internal similarity, and a Sy/Wr cluster (Sy357, Sy362, Sy364, Wr386) showing 98.1-98.7% internal similarity. Cross-group comparisons (By/Po vs Sy/Wr) showed slightly lower, yet still substantial, conservation (97.6-97.9%), representing the most divergent pairwise relationships in the dataset. Non-shared gene percentages (upper purple triangle) were consistently low, ranging from 1.2% to 3.0% of total gene content. Within-group comparisons revealed minimal non-shared content (1.2-2.2%), whereas cross-group comparisons (By/Po vs Sy/Wr) showed elevated values (2.7-3.0%). The highest non-shared percentage (3.0%) was observed between By354 and Sy364, consistent with these isolates belonging to different populations.

The orthogroup patterns and gene-sharing results demonstrate that, while all seven isolates maintain highly conserved core gene repertoires, two distinct groups are evident, differing modestly in accessory gene content: a By/Po group and a Sy/Wr group. This grouping is consistent with the population assignment results, which place By/Po in NA2 and Sy/Wr in NA1.

Functional annotation of By/Po (NA2) and Sy/Wr (NA1)-exclusive genes reveals distinct enrichment patterns.

To investigate whether group-exclusive genes contribute to differences in aggressiveness between isolate groups, we performed comprehensive functional annotation using eggNOG-mapper, which integrates COG, KEGG, and GO databases. Of the 286 exclusive genes in By/Po isolates, 199 (69.8%) received at least one functional annotation, while 87 (30.2%) remained

completely unannotated (Fig. 5C). In contrast, Sy/Wr isolates showed lower annotation rates, with only 112 of 200 exclusive genes (56%) receiving annotations and 88 (44%) remaining unannotated. This difference suggests that By/Po exclusive genes may harbor more conserved functional domains despite their group-specific presence.

GO term annotations were rare among exclusive genes, with only three genes (all from By/Po isolates) receiving GO annotations (Fig. 4D). Gene By355_g3680.t1 was particularly well-annotated with 46 GO terms spanning all three major categories: 30 biological process terms (including DNA replication, metabolic processes, and cellular biosynthesis), 6 molecular function terms (including RNA DNA polymerase activity and transferase activity), and 10 cellular component terms (primarily related to intracellular organelles and the nucleus). The other two genes (By354_g4118.t1 and By355_g4136.t1) had only four GO terms each, all related to cellular components (Fig. 5D). The predominance of biological process and molecular function terms in By355_g3680.t1 suggests it may play active metabolic or catalytic roles rather than serving purely structural functions.

COG functional category analysis revealed that "function unknown" [S] was the most abundant category in both groups, accounting for 66 genes (23.1%) in By/Po and 37 genes (18.5%) in Sy/Wr (Fig. 5E). However, several functional categories showed significant enrichment differences between groups. Amino acid transport and metabolism [E] was enriched in By/Po isolates (15 genes vs. 6 genes in Sy/Wr), as were replication, recombination, and repair [L] (14 vs. 6 genes). On the other hand, Sy/Wr was enriched in energy production and conversion [C] (12 vs 6 genes in By/Po) as well as in inorganic ion transport [P] which was only found in Sy/Wr (8 genes). Notably, secondary metabolite biosynthesis [Q] was represented in both groups (6 genes in By/Po, 6 genes in Sy/Wr), suggesting that group-exclusive genes may contribute to the production of specialized metabolites potentially involved in host interaction or competition.

Analysis of KEGG functional annotations revealed distinct patterns between the two groups (Fig. 5F). The heterokaryon incompatibility protein (K14454) was the most enriched function in Sy/Wr isolates, present in eight genes with high statistical significance ($P < 0.001$), whereas it was completely absent in By/Po isolates. The Sy/Wr group is also significantly enriched in cation/H⁺ antiporter genes (K03574, $P < 0.03$, 4 genes) and membrane protein genes (K14326, $P < 0.03$, 4 genes). In contrast, By/Po isolates were enriched for metabolic enzymes, including S-adenosylmethionine synthase (K03380, 4 genes), 3-hydroxyisobutyrate dehydrogenase (K00020, 3 genes), glutamate N-acetyltransferase (K00480, 3 genes), and serine hydroxymethyltransferase (K00675/K15466, 3 genes).

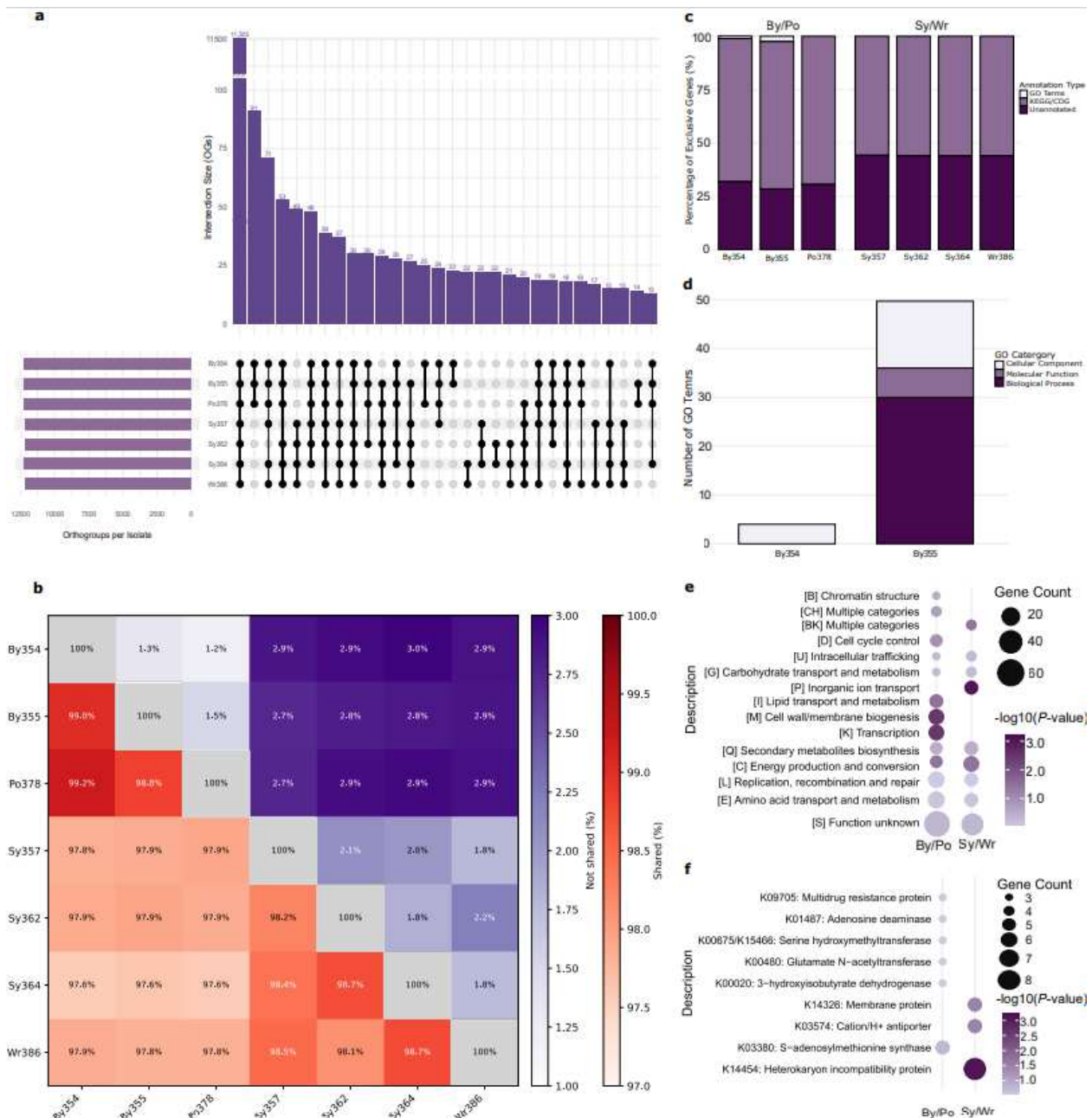


Figure 5. Exclusive orthogroups (OGs) and functional annotation of group-exclusive genes in cross-infectivity isolates. **a)** UpSet plot showing the top 30 orthogroup intersections across isolates. A large core set of 11,505 orthogroups is shared among all seven isolates, while smaller subsets are unique to specific combinations. **b)** Pairwise comparison of shared (lower red triangle) and non-shared (upper purple triangle) gene percentages among cross-infectivity isolates. Shared percentages were calculated as the proportion of orthogroup-assigned genes present in both isolates, normalized by the smaller isolate's gene set (values 97–100%). Non-shared percentages were calculated as the fraction of isolate-specific and unassigned genes relative to the combined gene totals for each pair (ranging from 1–3%). Gray diagonal boxes

denote self-comparisons, set to 100%. The restricted color scales (shared = 90–100%; non-shared = 1–3%) emphasize subtle but biologically relevant differences in genome content among isolates. **c)** Annotation coverage of exclusive genes by isolate. Genes were classified as having GO terms, KEGG/COG annotations, or remaining unannotated. By/Po isolates (barley/potato rotation) showed higher annotation rates (67-70%) compared to Sy/Wr isolates (soybean/winter rye rotation, 56%). **d)** GO term classification for the three genes with GO annotations. Both genes from By355 contained predominantly biological process terms (30 terms), with fewer molecular function (6 terms) and cellular component terms (10 and 4 terms for By355 and By354, respectively). **e)** Top 15 COG functional categories enriched in group-exclusive genes. Function unknown [S] was the most abundant category in both groups (66 genes in By/Po, 37 in Sy/Wr). Significant enrichment was observed for several categories, including transcription [K], cell wall/membrane biogenesis [M], and lipid transport and metabolism [I] in By/Po and energy production and conversion [C] and inorganic ion transport [P] in Sy/Wr. **f)** Top 15 annotated gene functions (KEGG) in group-exclusive genes. The heterokaryon incompatibility protein (K14454) was highly enriched in Sy/Wr (8 genes, $P < 0.001$), while S-adenosylmethionine synthase (K03380) and several other metabolic enzymes were exclusive to By/Po isolates.

Pathogenicity-related genes reside more on fast-speed subgenomes than slow-speed subgenomes.

Genome-wide fast- and slow-speed subgenome assignments for seven cross-infectivity isolates, inferred using fixed genomic windows along the PH-1 reference genome, are shown in Supplementary Fig. 3. Across the cross-infectivity isolates, fast- and slow-speed subgenome assignments were concordant in 74.2% of the genomic regions. Although the specific regions classified as fast or slow varied across isolates, most showed consistent subgenome assignment. Overall, the slow-speed subgenomes predominated in all isolates, comprising 62.3% of the By/Po group and 65.3% of the Sy/Wr group, while the remaining regions were classified as fast-speed subgenomes.

The fast subgenomes had a higher density of secreted proteins ($P = 0.000005$), carbohydrate-active enzymes ($P = 0.000007$), and effectors ($P = 0.000008$) compared to the slow subgenomes (Fig. 6A-C). Paired comparisons demonstrated clear within-isolate differences in subgenome-specific exclusive gene counts for both By/Po and Sy/Wr groups (Fig. 6D). In By/Po isolates, fast-speed subgenome counts were substantially higher than slow-speed subgenome counts ($P = 0.0004$), with an average increase of 49.3 genes. A significant fast–slow difference was also observed within Sy/Wr isolates ($P = 0.0212$), with fast-subgenome values exceeding slow-subgenome counts by an average of 4.5 genes. Between-group comparisons further supported pronounced differences. Total exclusive gene counts were significantly higher in By/Po than in Sy/Wr isolates ($P = 0.00006$). Fast-subgenome counts were likewise significantly greater in By/Po compared with Sy/Wr ($P = 0.00009$). In contrast, slow-subgenome counts did not differ significantly between By/Po and Sy/Wr isolates ($P = 0.3482$). Collectively, these analyses

indicate strong fast-speed subgenome enrichment in both By/Po and Sy/Wr isolates, with By/Po exhibiting markedly higher overall exclusive gene counts relative to Sy/Wr.

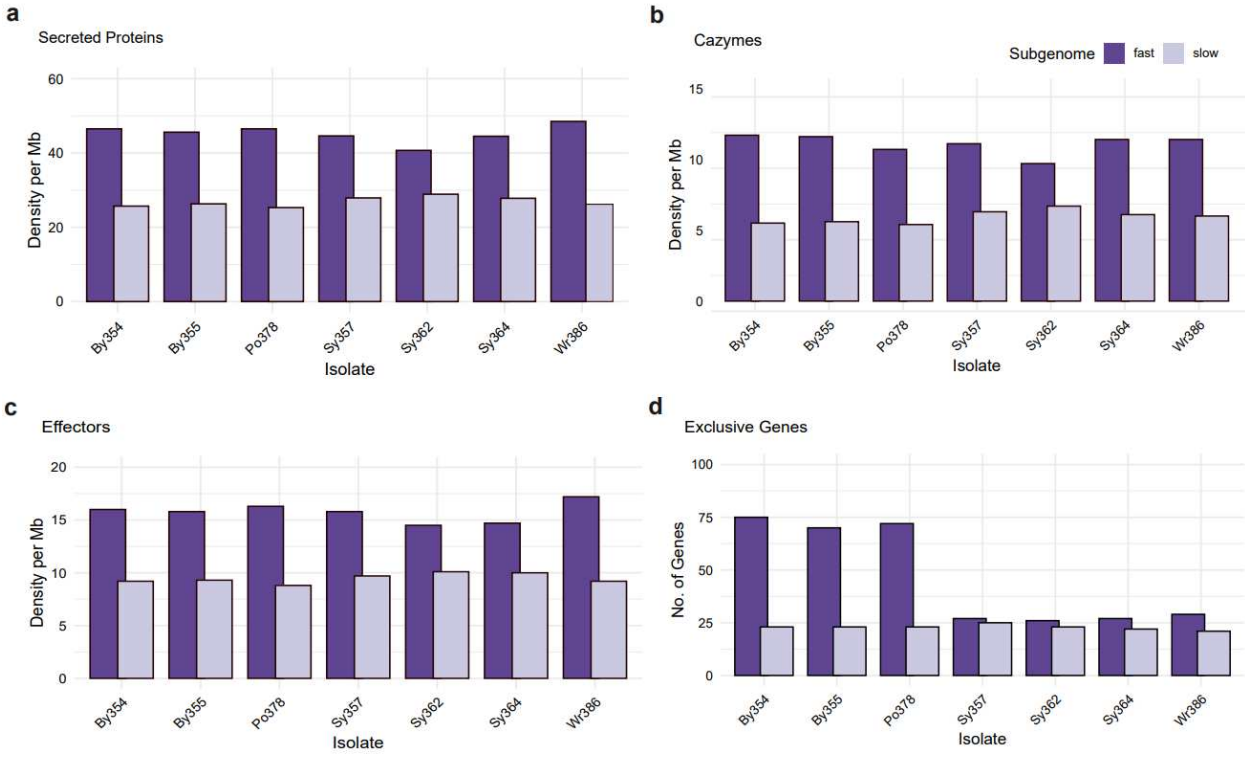


Figure 6. Genomic features and exclusive genes in fast- and slow-speed subgenomes of cross-infectivity isolates. Values between fast- and slow-subgenomes show significant differences for **A**) secreted proteins ($P = 0.000005$), **B**) carbohydrate-active enzymes ($P = 0.000007$), and **C**) effectors ($P = 0.000008$). No significant differences were detected between the By/Po and Sy/Wr groups in either the fast or slow subgenomes for any of the measured features: secreted proteins ($P = 0.3725$ and 0.1143), CAZymes ($P = 0.3725$ and 0.0571), or effectors ($P = 0.4755$ and 0.1536). **D**) Exclusive gene counts across fast and slow subgenomes for ByPo and SyWr isolate groups. Fast-subgenome counts were significantly higher than slow-subgenome counts within both By/Po ($P = 0.0004$) and Sy/Wr isolates ($P = 0.0212$). Between groups, ByPo isolates exhibited significantly greater total exclusive gene counts ($P = 0.00006$) and fast-subgenome counts ($P = 0.00009$) compared with SyWr isolates. Slow-subgenome counts did not differ significantly between groups ($P = 0.3482$).

In addition to performing functional annotation with COG and KEGG (Fig. 5E and 5F), we also queried the exclusive genes against the UniProt Swiss-Prot curated protein database using DIAMOND BLASTP ($1e-5$). Although there are more exclusive genes in By/Po than Sy/Wr isolates, most of them do not have homology matches in the database. A higher proportion of

genes with BLAST hits was found in Sy/Wr (38.5%) compared to By/Po isolates (24.8%) (Supplementary Fig. 4). For By/Po, 24 genes with functional assignments were identified, with 18 located in the fast-speed subgenome, four in the slow-speed subgenome, and two in either the fast or slow subgenomes depending on the isolate. In Sy/Wr, 20 genes with functional assignments were identified, including eight in the fast-speed subgenomes, five in either the fast or slow subgenomes depending on the isolate, and seven in the slow-speed subgenomes. All isolates within each group share the same functional profiles, except for three genes that show isolate-specific functions, such as ATP-dependent DNA helicase Q-like 3 unique to Po378, and Fe(II)/2-oxoglutarate-dependent dioxygenase *nvfI* and putative disease resistance protein *At3g14460* unique to Sy357 (Supplementary Table 7). Aside from these isolate-level differences, most genes in both groups encode enzymes, although their functions differ between By/Po and Sy/Wr. In the By/Po group, genes are primarily associated with secondary metabolite production, including methyltransferase *FGSG_00040*, 5-methylthioadenosine/S-adenosylhomocysteine deaminase, N-malonyltransferase *FDB2*, peroxisomal multifunctional enzyme type 2, and PKS-NRPS hybrid synthetase *cheA*. Most notably, one gene in this group encodes the trichothecene biosynthesis transcription regulator *TRI6*, and another encodes a trichothecene C-15 hydroxylase, neither of which is present in Sy/Wr. All of these genes are located in the fast-speed subgenomes of By/Po isolates. In contrast, genes in the Sy/Wr group include functions related to circadian rhythm regulation such as circadian oscillator component *FRQ/FRQ10*, sugar uptake with high-affinity glucose transporter *ght5*, as well as pathogen recognition through nucleotide-binding leucine-rich repeat (NLR)-like protein, all located in the fast-speed subgenomes. The oxidoreductase *BOA1* located in slow-speed subgenome of Sy/Wr isolates has functions that contribute to a plant pathogen's virulence (Dalmais et al., 2011). The Sy357-specific genes *Fe(II)/2-oxoglutarate-dependent dioxygenase nvfI* and putative disease resistance protein *At3g14460* are located in the slow-speed subgenome and are involved in the biosynthesis of the fungal metabolite novofumigatonin and in pathogen defense, respectively. Together, these patterns highlight functional differences between By/Po and Sy/Wr isolates, with distinct gene repertoires concentrated in the fast-speed subgenomes.

Discussion

F. graminearum is a cereal pathogen, but it is also known as a pathogen of other crops, such as potatoes (Ali et al., 2005; Estrada et al., 2010; Tiwari et al., 2020), soybeans (Ellis et al., 2011; Cruz et al., 2020; Okello et al., 2020), and recently hemp (Yulfo-Soto et al., 2022). Several studies linked this adaptive ability of *F. graminearum* to its fast-speed subgenome, which is enriched for pathogenicity-related genes (Sperschneider et al., 2015; Wang et al., 2017). The ability of this pathogen to infect several crops and the evolution of its fast-speed genome have been reported separately by different papers, but ours is the first study to demonstrate the

association between cross-infectivity patterns, mycotoxin profiles, and genomic variation in *Fusarium graminearum*.

In this study, we sequenced and assembled seven high-quality *F. graminearum* genomes from barley, potato, soybean, and winter rye using both long- and short-read sequences. Polished assemblies are highly contiguous with 4-5 contigs and >99.2% completeness. The assemblies generated in this study will serve as a valuable resource to support other genomic studies involving *F. graminearum*. We cross-inoculated these seven isolates onto barley, potatoes, and soybeans. All isolates are capable of infecting their original crops and barley, confirming that they are cereal pathogens. However, we found *F. graminearum* isolates from barley and potato are more aggressive than those from other sources, particularly when inoculated to potatoes. Among all isolates, By355 is the most aggressive.

Multiple complementary analyses were applied to the *F. graminearum* isolates, including genome-wide comparisons, cross-infectivity tests, mycotoxin profiling, population assignment, and orthologs-based analyses. Each of these approaches supports the classification of isolates into two distinct groups. These comprise the more aggressive By/Po-NA2 group (By354, By355, and Po378) and the less aggressive Sy/Wr-NA1 group (Sy357, Sy362, Sy364, and Wr386).

Mycotoxin analysis separates our isolates into two major chemotype groups. The By/Po isolates (By354, By355, Po378) form a strong 3-ADON group, producing high levels of both DON and 3-ADON. In contrast, the Sy/Wr isolates (Sy357, Sy362, Sy364, Wr386) group as 15-ADON producers. This chemotype grouping is further supported by the TRI8 analysis, which shows that By/Po isolates have TRI8 sequences similar to those of 3-ADON reference sequences, whereas Sy/Wr isolates match 15-DON reference sequences. Our study indicated that By/Po, which are 3-ADON producers, are more aggressive than Sy/Wr, which are 15-ADON producers. An initial study by Goyal et al. (2024) tested recombinants from a cross of NA1-15ADON x NA2-3ADON and revealed that 3-ADON progenies do not exhibit greater aggressiveness or toxigenicity than 15-ADON progenies. This suggests that factors other than chemotype may be responsible for aggressiveness. As Goyal et al. (2024) only tested aggressiveness in wheat, extending chemotype-aggressiveness tests of the progenies in non-cereal crops would further distinguish the role of chemotype along with input from genomic regions and elements on aggressiveness and host-adaptivity. Only a few studies have explored *F. graminearum* chemotypes outside of cereals. In potatoes, Delgado et al. (2010) detected DON, NIV, 3-ADON, and 15-ADON in rotted tuber tissues inoculated with multiple *F. graminearum* isolates, with DON, followed by NIV, present at higher levels than 3-ADON and 15-ADON. The *F. graminearum* isolates used in that study were obtained from potato, wheat, and sugarbeet, and 11 out of 14 were classified as DON chemotypes. In soybeans, Barros et al. (2012) found that most *F. graminearum* isolates from pre-harvest soybean samples were 15-ADON. In another study, Ellis and Munkvold (2014) examined 38 *F. graminearum* isolates from soybeans and, contrary to their hypothesis that 3-

ADON might be linked to the host shift to soybeans in North America, found that all isolates were 15-ADON. Their results suggested that the rise of soybean infections was not driven by the introduction of 3-ADON genotypes. Our findings are consistent with these studies in showing that *F. graminearum* infects both potatoes and soybeans. However, we add important insights: (1) potato infections in our dataset are dominated by the strong 3-ADON producers, (2) the 3-ADON producers By355 and Po378 are also aggressive on soybean, and (3) all soybean isolates in this study also belong to the 15-ADON group, with Sy357 producing the highest 15-ADON and DON levels, likely contributing to its high aggressiveness in barley and soybean but not in potato. The trichodiene synthase gene (*Tri5*) was previously disrupted in the fungal strain GZ3639 to generate GZT40, a strain incapable of producing DON (Bai et al., 2002). This DON-nonproducing strain of *F. graminearum* initiates infection but fails to facilitate disease progression in wheat, suggesting that DON is crucial for pathogen spread from one spike to another, but is not a prerequisite for the initial infection by the fungus. In potato tubers, although *F. graminearum* was recovered from the rotted tissue and surrounding areas, Delgado et al. (2010) reported that DON was detected in the rotted tissue rather than in the surrounding tissue. It will be interesting to determine whether knocking out the *Tri5* gene in the Po378 isolate decreases infection or symptom development in potatoes.

Population assignment of the cross-infectivity isolates shows clustering of the By/Po group within NA2 and Sy/Wr within NA1. Variation within By/Po was lower compared to Sy/Wr, which indicates barley and potato isolates are genetically more similar compared to soybean and winter rye isolates. We have established here that By/Po are NA2 and 3-ADON producers while Sy/Wr are NA1 and 15-ADON producers. This clustering is mirrored in the symptoms on potato, where the NA2/3-ADON group caused higher severity than the NA1/15-ADON group, but there are no clear differences between these groups in soybeans and barley. For example, Sy357 exhibits high severity on soybean and barley but not on potato. At the same time, By355 and Po378 are also aggressive on soybeans. There are conflicting reports on the aggressiveness of *F. graminearum* populations and chemotypes. In spring wheat, Puri and Zhong (2010) reported that the 3-ADON chemotype was both more aggressive and produced more DON, a finding later supported by RNA-seq data (Puri et al., 2016). Similarly, Amarasinghe et al. (2019) observed higher FHB severity and *Fusarium*-damaged kernels (FDK) for 3-ADON strains. A recent winter wheat study also indicated increased aggressiveness, higher FDK, and greater DON production for 3-ADON strains compared to 15-ADON strains (Tamburic-Ilincic, 2025). However, this latter observation was only statistically significant in one of the four growing seasons examined (2011, but not 2012, 2023, or 2024). Conversely, other studies have found no significant difference in aggressiveness between the two chemotypes. Specifically, von der Ohe et al. (2010) found no significant difference in FHB indices between 3-ADON and 15-ADON isolates in spring wheat. Mueller et al. (2021) likewise reported no significant differences in aggressiveness between *F. graminearum* chemotypes in Wisconsin. A potential reason for the conflicting reports is that increased aggressiveness does not segregate with chemotype, as shown by Goyal et al.

(2024), but only co-occurs in most field isolates, and that aggressiveness may vary substantially within chemotypes, depending on which alleles a strain carries at other loci throughout its genome. Despite this variability in aggressiveness findings, the consensus across multiple studies is that 3-ADON *F. graminearum* isolates consistently produce more DON than the 15-ADON isolates (Puri and Zhong, 2010; von der Ohe et al., 2010; Amarasinghe et al., 2019; Mueller et al., 2021). However, these observations are confined to wheat, and no comparable studies have been conducted in barley or potato. This study addressed this gap. The results of our barley inoculations show that the 3-ADON chemotypes By354, By355, and Po378 are not the most aggressive in barley, but they produced higher DON levels. Most notably, the distinct separation between aggressiveness of 3-ADON/NA2 versus 15-ADON/NA1 isolates is particularly evident in potato inoculations, where By/Po isolates caused greater severity than Sy/Wr isolates.

All seven isolates share a large set of 11,505 orthogroups, indicating conserved functions. However, comparing non-shared genes reveals two groups, By/Po and Sy/Wr, which support the classification results based on aggressiveness, SNPs, and chemotypes. We examined the functions of these non-shared genes and found that a significant proportion (30-44%) are unannotated or have general "function unknown" annotations. These unannotated genes may indicate that many group-exclusive genes are lineage-specific changes or rapidly evolving genes lacking homology to characterized proteins in public databases. Genes with known functions were examined and showed distinct functional profiles between the By/Po and Sy/Wr groups, suggesting that these exclusive genes may contribute to different adaptive strategies. The By/Po group exhibits a molecular profile rich in pathways associated with transcription, cell wall and membrane biogenesis, and lipid transport and metabolism. This composition may provide enhanced adaptability, facilitating effective host colonization and improved survival against environmental stressors. Specifically, in *F. graminearum*, crucial transcriptional regulation affects key biological processes such as spore germination and host invasion (Brauer et al., 2020), thereby possibly contributing to the advantageous characteristics of the By/Po group. Conversely, the Sy/Wr group is significantly enriched in KEGG heterokaryon incompatibility protein (K14454). This protein triggers a programmed cell death reaction that compartmentalizes and destroys the fusion cell. It is unclear, however, how or if this relates to aggressiveness, but one explanation is that these isolates may use this strategy to prevent genome exploitation (Saupe, 2000; Daskalov et al., 2017). For example, in *F. graminearum*, mitogen-activated protein kinase gene *MGV1* mutants (*mgv1* mutants) exhibited self-incompatibility, resulting in reduced virulence. *MGV1* is known to be involved in various processes, including sexual reproduction, plant infection, and cell wall integrity (Hou et al., 2022). Our findings indicate that while *F. graminearum* has conserved regions (core), a small part of its genome (accessory) is variable and may influence the fungal pathogen's ability to infect specific crops.

The fast-evolving subgenome of *F. graminearum* has been credited with its ability to adapt and infect, as evidenced by higher proportions of genes related to pathogenicity compared to the

slow-evolving subgenome. The fast-speed subgenome of *F. graminearum* has a higher variant frequency, while the slow-speed subgenome has a lower variant frequency (Wang et al., 2017). Here, we delineate the fast- and slow-subgenomes based on SNP variation and examined protein-coding genes, secreted proteins, and carbohydrate-active enzymes. We found that across isolates, these genes are more abundant in the fast-speed subgenome than in the slow-speed subgenomes, consistent with the findings of Wang et al. (2017). However, we did not observe a significant difference in the number of these genes between the By/Po and Sy/Wr groups. Additionally, the By/Po group has more exclusive genes in the fast-subgenome compared with the Sy/Wr group. These genes may represent group-specific functions that contribute to aggressiveness, particularly in potato infections. This difference is clear in the protein homology results, where By/Po genes are primarily associated with secondary metabolite production, while Sy/Wr genes are associated with circadian rhythms, pathogen recognition, and sugar uptake. However, because gene prediction and functional annotation were guided by the PH-1 reference genome, which belongs to the NA1 population, some degree of ascertainment bias cannot be excluded. However, such reference-based bias would be expected to favor NA1 (Sy/Wr) and potentially underrepresent divergent gene content in NA2 (By/Po). The observed enrichment of fast-subgenome exclusive genes in By/Po, an NA2 group, therefore suggests that this pattern is unlikely to be solely driven by annotation bias. However, additional validation would be needed to fully establish its biological significance.

Analyses of transposable elements (TEs) show that the By/Po group has a higher count of the *Tc Mariner* repeat class than the Sy/Wr group. The *Tc Mariner* DNA transposon superfamily is known to be the most widespread and plentiful in sequenced fungal genomes (Muszewska et al., 2017). This observation is consistent with the By/Po group, which exhibits high counts (72-89), but not with the Sy/Wr group, which shows very low counts (1-2). This repeat class was previously reported in the plant pathogen *Fusarium oxysporum*, where it was shown to affect gene structure and function. Specifically, it can cause gene inactivation through insertion, cause nucleotide sequence diversification via imprecise excisions, and potentially lead to chromosomal rearrangements (Daboussi et al., 1994). This influence on the host genome might explain its presence across a wide variety of hosts, although the exact mechanism underlying this broad distribution remains unclear (Duperyon et al., 2020). Additionally, the By/Po group has one *hAT* count, which is absent in the other group. TEs from the *hAT* superfamily are prevalent in fungal genomes and have been implicated in pathogen adaptation. A notable example is the fungal pathogen *Fusarium oxysporum*, which underwent rapid adaptation after serial passage through tomato plants. Mutational changes in the evolved lines were largely driven by TE insertions with a single, non-autonomous *hAT*-type TE accounting for 63% of TE-associated events, underscoring its crucial role in driving mutations (Lopez-Diaz et al., 2025). Furthermore, *Kolobok* repeats were only found in By355, the most aggressive isolate, with 24 counts. Research specifically focused on *Kolobok* elements is limited, with only one report detailing their amplification in the clawed frog, *Xenopus*, suggesting their involvement in complex

contributions to T2-MITE transposition (Hikosaka et al., 2018). Genomic analyses also found no evident differences between By/Po and Sy/Wr in gene model counts, but the barley isolate By354 consistently had the highest numbers of secreted proteins, effectors, and CAZymes. Similar to gene models, secondary metabolites are very similar across all isolates.

The findings of our study have important implications for diverse cropping systems. The adaptive capability of *F. graminearum*, as established in this research, presents a risk to subsequent crops. Additionally, the selection of the preceding crop can also affect the resulting disease pressure. For example, low FHB incidence in wheat has been observed when it is planted after potato, canola, pasture (Vogelsang et al., 2019), and soybeans (Dill-Macky and Jones, 1997). Soybeans as a preceding crop may mitigate the risk of infection by *F. graminearum* and *F. poae* (Vogelsang et al., 2019), possibly due to lower residue levels than those of other crops such as maize and wheat (Dill-Macky and Jones, 1997). However, our research also identified a potential risk to soybean cultivation posed by isolate Sy357, which showed greater aggressiveness against both barley and soybean.

Residues left after cereal crop harvests play a crucial role in maintaining disease pressure throughout the seasons. *Fusarium graminearum* has been frequently detected in crop residues, with incidences of 50% in wheat, 40% in corn, and 26% in soybeans (Dill-Macky and Jones, 1997). Residue management may have a stronger influence on disease persistence than tillage practices alone, as reported by Maiorano et al. (2008), particularly when residues are derived from high-biomass crops such as maize. Further supporting this is an extensive 8-year wheat survey, which reported *F. graminearum* as the dominant *Fusarium* species, accounting for 63% of all detections, with yearly fluctuations likely reflecting changes in environmental conditions and crop management (Vogelsang et al., 2019). Collectively, these results highlight the importance of understanding the field-based capacity of *F. graminearum* to survive and cross-infect across crop rotation cycles, which may have direct implications for residue and rotation management strategies.

Here, we report that the more aggressive *F. graminearum* isolates (NA2) are genetically distinct from the less aggressive ones (NA1), exhibiting greater genetic similarity among themselves. This aggressiveness, particularly for the potato infections, is associated with the NA2 population/3-ADON chemotype and with the exclusive genes in the fast subgenome. Our findings also show that *F. graminearum* can infect various crops in rotation, posing a hidden hazard across several agricultural systems. Expanding *F. graminearum* studies to non-cereal crops will clarify how host context shapes pathogen behavior beyond traditional cereal systems. To our knowledge, our study is the first to link cross-infectivity patterns and mycotoxin profiles with genomic variation in *F. graminearum* across crops, marking an important step toward a more comprehensive understanding of its adaptability.

Data Availability

Data for this study were submitted to NCBI under BioProject PRJNA1425175 (<https://www.ncbi.nlm.nih.gov/sra/PRJNA1425175>). Nanopore and Illumina sequence reads were deposited in NCBI Sequence Read Archive carrying Biosample IDs SAMN55409815 to SAMN55409821. The Whole Genome project has been deposited at DDBJ/ENA/Genbank under accession JBVBER000000000 (By354), JBVBEQ000000000 (By355), JVBEP000000000 (Po378), JVBEO000000000 (Sy357), JVBEN000000000 (Sy362), JVBEM000000000 (Sy364), JVBEL000000000 (Wr386). The version described in this paper is version JBVBER010000000 (By354), JBVBEQ010000000 (By355), JVBEP010000000 (Po378), JVBEO010000000 (Sy357), JVBEN010000000 (Sy362), JVBEM010000000 (Sy364), JVBEL010000000 (Wr386).

Funding

This material is based upon work that is supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, under agreement number 2023-38640-39573 through the North Central Region SARE program under project number GNC24-399. USDA is an equal opportunity employer and service provider. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

Author contributions

A.R.N and T.B. conceptualized the idea and obtained funding for this project. T.B. supervised the study. A.R.N designed and conducted the experiments, performed formal analysis, validation, and visualization, and wrote the first draft of the manuscript. V.R.V. and H.N. assisted in the investigation and experimental design. H.N. helped with visualization. J.S. assembled the draft genome. Z.J. contributed to the mycotoxin analysis. M.O. reviewed the statistical analysis. J.S., Z. J., M.O., U.G., C.T., G.S., and T.B. contributed with review revisions. C.T. and T.B. assisted with data interpretation and writing. All authors reviewed and approved the final version of the manuscript.

Acknowledgment

We thank Dr. Richard Webster for the Williams 82 soybean seeds and Dr. Andrew Friskop for the winter rye samples. We also thank Dr. Febina Mathew and Nitha Rafi for the *F. graminearum* soybean isolates. We appreciate Jatinder Singh for his help in the draft genome assembly. We are grateful to Kathy Christianson of NDSU DON Testing Laboratory for Wheat and Barley for accommodating our samples for mycotoxin analysis, as well as to the NDSU Jack Dalrymple Agricultural Research Complex for the space to grow our plants. This work used resources of the Center for Computationally Assisted Science and Technology (CCAST) at North Dakota State University, which were made possible in part by NSF MRI Award No. 2019077. Contribution no. 26-123-J from the Kansas Agricultural Experiment Station.

Supplementary Files

<https://docs.google.com/spreadsheets/d/1c8yJ5R6krEz15uwuzWW8Tbso3oy4n2eS/edit?gid=1269199894#gid=1269199894>

References:

1. Alexander, N.J., McCormick, S.P., Waalwijk, C., van der Lee, T. and Proctor, R.H., 2011. The genetic basis for 3-ADON and 15-ADON trichothecene chemotypes in *Fusarium*. *Fungal Genetics and Biology*, 48(5), pp.485-495.
2. Ali, S., Rivera, V. V. & Secor, G. A. First report of *Fusarium graminearum* causing dry rot of potato in North Dakota. *Plant Dis.* **89**, 105 (2005).
3. Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. Basic local alignment search tool. *J. Mol. Biol.* **215**, 403–410 (1990).
4. Amarasinghe, C., Sharanowski, B. & Fernando, W. D. Molecular phylogenetic relationships, trichothecene chemotype diversity and aggressiveness of strains in a global collection of *Fusarium graminearum* species. *Toxins* **11**, 263 (2019).
5. Andrews, S. FastQC: a quality control tool for high throughput sequence data. <http://www.bioinformatics.babraham.ac.uk/projects/fastqc/> (2010).
6. Bai, G. H., Desjardins, A. E. & Plattner, R. D. Deoxynivalenol-nonproducing *Fusarium graminearum* causes initial infection, but does not cause disease spread in wheat spikes. *Mycopathologia* **153**, 91–98 (2002).
7. Bandi, V. & Gutwin, C. Interactive exploration of genomic conservation. *Graphics Interface 2020* (2020).
8. Barros, G., Zanon, M. A., Abod, A., Oviedo, M. S., Ramirez, M. L., Reynoso, M. M., Torres, A. & Chulze, S. Natural deoxynivalenol occurrence and genotype and chemotype determination of a field population of the *Fusarium graminearum* complex associated with soybean in Argentina. *Food Addit. Contam. Part A* **29**, 293–303 (2012).
9. Bilgi, V. N., Bradley, C. A., Mathew, F. M., Ali, S. & Rasmussen, J. B. Root rot of dry edible bean caused by *Fusarium graminearum*. *Plant Health Prog.* **12**, 14 (2011).
10. Blin, K., Shaw, S., Kloosterman, A. M., Charlop-Powers, Z., van Wezel, G. P., Medema, M. H. & Weber, T. antiSMASH 6.0: improving cluster detection and comparison capabilities. *Nucleic Acids Res.* **49**, 29–35 (2021).
11. Brauer, E. K., Subramaniam, R. & Harris, L. J. Regulation and dynamics of gene expression during the life cycle of *Fusarium graminearum*. *Phytopathology* **110**, 1368–1374 (2020).
12. Walker, B. J. *et al.* Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. *PLoS ONE* **9**, e112963 (2014).
13. Brůna, T., Hoff, K. J., Lomsadze, A., Stanke, M. & Borodovsky, M. BRAKER2: automatic eukaryotic genome annotation with GeneMark-EP+ and AUGUSTUS supported by a protein database. *NAR Genomics Bioinform.* **3**, lqaa108 (2021).

14. Bruna, T., Lomsadze, A. & Borodovsky, M. GeneMark-ETP: automatic gene finding in eukaryotic genomes in consistency with extrinsic data. *BioRxiv* (2023).
15. Bolger, A. M., Lohse, M. & Usadel, B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**, 2114–2120 (2014).
16. Bushnell, B. BBTools software package. <http://sourceforge.net/projects/bbmap> (2014).
17. Buchfink, B., Xie, C. & Huson, D. H. Fast and sensitive protein alignment using DIAMOND. *Nat. Methods* **12**, 59–60 (2015).
18. Buchfink, B., Reuter, K. & Drost, H. G. Sensitive protein alignments at tree-of-life scale using DIAMOND. *Nat. Methods* **18**, 366–368 (2021).
19. Cantalapiedra, C. P., Hernández-Plaza, A., Letunic, I., Bork, P. & Huerta-Cepas, J. eggNOG-mapper v2: functional annotation, orthology assignments, and domain prediction at the metagenomic scale. *Mol. Biol. Evol.* **38**, 5825–5829 (2021).
20. Cai, L., Chen, R., Zi, Y. & Zhao, C. First report of *Panax notoginseng* wilt disease caused by *Fusarium graminearum* in China. *Plant Dis.* **107**, 3306 (2023).
21. Chen, Y., Zhang, Y., Wang, A. Y., Gao, M. & Chong, Z. Accurate long-read de novo assembly evaluation with Inspector. *Genome Biol.* **22**, 1–21 (2021).
22. Chen, Y., Nie, F., Xie, S. Q., Zheng, Y. F., Dai, Q., Bray, T., Wang, Y. X., Xing, J. F., Huang, Z. J., Wang, D. P., He, L. J., Luo, F., Wang, J. X., Liu, Y. Z. & Xiao, C. L. Efficient assembly of nanopore reads via highly accurate and intact error correction. *Nat. Commun.* **12**, 60 (2021).
23. Cingolani, P., Platts, A., Wang, L. L., Coon, M., Nguyen, T., Wang, L., Land, S. J., Lu, X. & Ruden, D. M. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of *Drosophila melanogaster* strain w1118; iso-2; iso-3. *Fly* **6**, 80–92 (2012).
24. Cock, P. J., Antao, T., Chang, J. T., Chapman, B. A., Cox, C. J., Dalke, A., Friedberg, I., Hamelryck, T., Kauff, F., Wilczynski, B. & De Hoon, M. J. Biopython: freely available Python tools for computational molecular biology and bioinformatics. *Bioinformatics* **25**, 1422 (2009).
25. Cruz, D. R., Leandro, L. F. S., Mayfield, D. A., Meng, Y. & Munkvold, G. P. Effects of soil conditions on root rot of soybean caused by *Fusarium graminearum*. *Phytopathology* **110**, 1693–1703 (2020).
26. Daboussi, M. J. & Langin, T. Transposable elements in the fungal plant pathogen *Fusarium oxysporum*. *Genetica* **93**, 49–59 (1994).
27. Dalmais, B., Schumacher, J., Moraga, J., Le Pecheur, P., Tudzynski, B., Collado, I.G. & Viaud, M.. The *Botrytis cinerea* phytotoxin botcinic acid requires two polyketide synthases for production and has a redundant role in virulence with botrydial. *Molecular Plant Pathology*, **12**(6), 564–579 (2011).
28. Danecek, P., Bonfield, J. K., Liddle, J., Marshall, J., Ohan, V., Pollard, M. O., Whitwham, A., Keane, T., McCarthy, S. A., Davies, R. M. & Li, H. Twelve years of SAMtools and BCFtools. *Gigascience* **10**, giab008 (2021).

29. Daskalov, A., Heller, J., Herzog, S., Fleißner, A. & Glass, N. L. Molecular mechanisms regulating cell fusion and heterokaryon formation in filamentous fungi. *Microbiol. Spectr.* **5**, 10.1128 (2017).
30. Delgado, J. A., Schwarz, P. B., Gillespie, J., Rivera-Varas, V. V. & Secor, G. A. Trichothecene mycotoxins associated with potato dry rot caused by *Fusarium graminearum*. *Phytopathology* **100**, 290–296 (2010).
31. Dhakal, U., Yue, W., Leslie, J. F. & Toomajian, C. Population genomics of *Fusarium graminearum* isolates from the Americas. *Fungal Genet. Biol.* **174**, 103924 (2024).
32. Diaz, J., Garcia, J., Lara, C., Hutmacher, R. B., Ulloa, M., Nichols, R. L. & Ellis, M. L. Characterization of current *Fusarium oxysporum* f. sp. *vasinfectum* isolates from cotton in the San Joaquin Valley of California and Lower Valley El Paso, Texas. *Plant Dis.* **105**, 1898–1911 (2021).
33. Dill-Macky, R. & Jones, R. K. The effect of previous crop residues and tillage on *Fusarium* head blight of wheat. *Plant Dis.* **84**, 71–76 (2000).
34. Dupeyron, M., Baril, T., Bass, C. & Hayward, A. Phylogenetic analysis of the Tc1/mariner superfamily reveals the unexplored diversity of pogo-like elements. *Mob. DNA* **11**, 21 (2020).
35. Ellis, M. L., Broders, K. D., Paul, P. A. & Dorrance, A. E. Infection of soybean seed by *Fusarium graminearum* and effect of seed treatments on disease under controlled conditions. *Plant Dis.* **95**, 401–407 (2011).
36. Ellis, M. L. & Munkvold, G. P. Trichothecene genotype of *Fusarium graminearum* isolates from soybean (*Glycine max*) seedling and root diseases in the United States. *Plant Dis.* **98**, 1012 (2014).
37. Emms, D. M. & Kelly, S. OrthoFinder: solving fundamental biases in whole genome comparisons dramatically improves orthogroup inference accuracy. *Genome Biol.* **16**, 157 (2015).
38. Emms, D. M. & Kelly, S. OrthoFinder: phylogenetic orthology inference for comparative genomics. *Genome Biol.* **20**, 1–14 (2019).
39. Estrada, R., Jr., Gudmestad, N. C., Rivera, V. V. & Secor, G. A. *Fusarium graminearum* as a dry rot pathogen of potato in the USA: prevalence, comparison of host isolate aggressiveness and factors affecting aetiology. *Plant Pathol.* **59**, 1114–1120 (2010).
40. Fernandez, M. R. Recovery of *Cochliobolus sativus* and *Fusarium graminearum* from living and dead wheat and nongramineous winter crops in southern Brazil. *Can. J. Bot.* **69**, 1900–1906 (1991).
41. Flynn, J. M., Hubley, R., Goubert, C., Rosen, J., Clark, A. G., Feschotte, C. & Smit, A. F. RepeatModeler2 for automated genomic discovery of transposable element families. *Proc. Natl Acad. Sci. USA* **117**, 9451–9457 (2020).
42. Gabriel, L., Hoff, K. J., Bruna, T., Borodovsky, M. & Stanke, M. TSEBRA: transcript selector for BRAKER. *BMC Bioinform.* **22**, 1–12 (2021).

43. Gabriel, L., Bruna, T., Hoff, K. J., Ebel, M., Lomsadze, A., Borodovsky, M. & Stanke, M. BRAKER3: fully automated genome annotation using RNA-seq and protein evidence with GeneMark-ETP, AUGUSTUS, and TSEBRA. *Genome Res.* **34**, 769–777 (2024).
44. Garma, N., El-Shetehy, M., Sayari, M., Gharbi, Y., Henriquez, M. A., Wang, X., McLaren, D. & Daayf, F. Comparing *Fusarium* inoculation protocols for soybean root rot infection. *J. Plant Pathol.* 1–10 (2025).
45. Goswami, R. S. & Kistler, H. C. Heading for disaster: *Fusarium graminearum* on cereal crops. *Mol. Plant Pathol.* **5**, 515–525 (2004).
46. Goyal, S., Farman, M., Proctor, R. H., McMaster N., Schmale D. J., Van Sanford., D., & Vaillancourt, L. J. Recombination Analysis Reveals 3ADON Does Not Confer Higher Levels of Aggressiveness or Toxigenicity than 15ADON [Abstract]. p. 57 In: *Proceedings of the 2024 National Fusarium Head Blight Forum*. Austin, Texas, December 8-10, 2024.
47. Gotoh, O. A space-efficient and accurate method for mapping and aligning cDNA sequences onto genomic sequence. *Nucleic Acids Res.* **36**, 2630–2638 (2008).
48. Yu, G., Smith, D., Zhu, H., Guan, Y. & Lam, T. T.-Y. ggtree: an R package for visualization and annotation of phylogenetic trees with their covariates and other associated data. *Methods Ecol. Evol.* **8**, 28–36 (2017).
49. Yu, G., Lam, T. T.-Y., Zhu, H. & Guan, Y. Two methods for mapping and visualizing associated data on phylogeny using ggtree. *Mol. Biol. Evol.* **35**, 3041–3043 (2018).
50. Guindon, S., Dufayard, J. F., Lefort, V., Anisimova, M., Hordijk, W. & Gascuel, O. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Syst. Biol.* **59**, 307–321 (2010).
51. Hanson, L. E. Fusarium yellowing of sugar beet caused by *Fusarium graminearum* from Minnesota and Wyoming. *Plant Dis.* **90**, 686 (2006).
52. Hikosaka, A. & Konishi, S. Multiple massive domestication and recent amplification of Kolobok superfamily transposons in the clawed frog *Xenopus*. *Zool. Lett.* **4**, 17 (2018).
53. Hou, Z., Xue, C., Peng, Y., Katan, T., Kistler, H. C. & Xu, J. R. A mitogen-activated protein kinase gene (MGV1) in *Fusarium graminearum* is required for female fertility, heterokaryon formation, and plant infection. *Mol. Plant Microbe Interact.* **15**, 1119–1127 (2002).
54. Hoff, K. J., Lange, S., Lomsadze, A., Borodovsky, M. & Stanke, M. BRAKER1: unsupervised RNA-Seq-based genome annotation with GeneMark-ET and AUGUSTUS. *Bioinformatics* **32**, 767–769 (2016).
55. Hoff, K. J., Lomsadze, A., Borodovsky, M. & Stanke, M. Whole-genome annotation with BRAKER. *Methods Mol. Biol.* **1962**, 65–95 (2019).
56. Hoang, D. T., Chernomor, O., Von Haeseler, A., Minh, B. Q. & Vinh, L. S. UFBoot2: improving the ultrafast bootstrap approximation. *Mol. Biol. Evol.* **35**, 518–522 (2018).
57. Huerta-Cepas, J., Szklarczyk, D., Heller, D., Hernández-Plaza, A., Forslund, S. K., Cook, H., Mende, D. R., Letunic, I., Rattei, T., Jensen, L. J. & von Mering, C. eggNOG 5.0: a

- hierarchical, functionally and phylogenetically annotated orthology resource based on 5090 organisms and 2502 viruses. *Nucleic Acids Res.* **47**, D309–D314 (2019).
58. Iwata, H. & Gotoh, O. Benchmarking spliced alignment programs including Spaln2, an extended version of Spaln that incorporates additional species-specific features. *Nucleic Acids Res.* **40**, e161 (2012).
59. Jin, Z., Solanki, S., Borowicz, P., Balwin, T., Xu, M. & Schwarz, P. Aberrant behavior of deoxynivalenol production in the malting of *Fusarium* head blight infected barley: mycotoxin accumulation and hyphal localization in single kernels. *J. Am. Soc. Brew. Chem.* **82**, 214–224 (2024).
60. Käll, L., Krogh, A. & Sonnhammer, E. L. A combined transmembrane topology and signal peptide prediction method. *J. Mol. Biol.* **338**, 1027–1036 (2004).
61. Kalyanamoorthy, S., Minh, B. Q., Wong, T. K. F., von Haeseler, A. & Jermini, L. S. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat. Methods* **14**, 587–589 (2017).
62. Katoh, K. & Standley, D. M. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol. Biol. Evol.* **30**, 772–780 (2013).
63. Kelly, A. C. & Ward, T. J. Population genomics of *Fusarium graminearum* reveals signatures of divergent evolution within a major cereal pathogen. *PLoS ONE* **13**, e0194616 (2018).
64. Kim, D., Paggi, J. M., Park, C., Bennett, C. & Salzberg, S. L. Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. *Nat. Biotechnol.* **37**, 907–915 (2019).
65. Krzywinski, M., Schein, J., Birol, I., Connors, J., Gascoyne, R., Horsman, D., Jones, S. J. & Marra, M. A. Circos: an information aesthetic for comparative genomics. *Genome Res.* **19**, 1639–1645 (2009).
66. Kolde, R. Pheatmap: pretty heatmaps. R package version 1.0.12 (2019).
67. Kovaka, S., Zimin, A. V., Pertea, G. M., Razaghi, R., Salzberg, S. L. & Pertea, M. Transcriptome assembly from long-read RNA-seq alignments with StringTie2. *Genome Biol.* **20**, 1–13 (2019).
68. Laurent, B., Palaikostas, C., Spataro, C., Moinard, M., Zehraoui, E., Houston, R. D. & Foulongne-Oriol, M. High-resolution mapping of the recombination landscape of the phytopathogen *Fusarium graminearum* suggests two-speed genome evolution. *Mol. Plant Pathol.* **19**, 341–354 (2018).
69. Leplat, J., Friberg, H., Abid, M. & Steinberg, C. Survival of *Fusarium graminearum*, the causal agent of Fusarium head blight. A review. *Agron. Sustain. Dev.* **33**, 97–111 (2013).
70. Letunic, I. & Bork, P. Interactive Tree of Life (iTOL) v6: recent updates to the phylogenetic tree display and annotation tool. *Nucleic Acids Res.* **52**, W78–W82 (2024).
71. Li, H. Minimap2: pairwise alignment for nucleotide sequences. *Bioinformatics* **34**, 3094–3100 (2018).

72. Li, H. & Durbin, R. Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics* **25**, 1754–1760 (2009).
73. Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R. & 1000 Genome Project Data Processing Subgroup. The sequence alignment/map format and SAMtools. *Bioinformatics* **25**, 2078–2079 (2009).
74. Liu, Z., No, E. G., Clement, C. D., He, P. & Shan, L. Isolation of high-molecular-weight (HMW) DNA from *Fusarium oxysporum* for long-read sequencing. *Methods Mol. Biol.* **2391**, 13–20 (2022).
75. López Díaz, C., Ayhan, D. H., Rodríguez López, A., Gómez Gil, L., Ma, L. J. & Di Pietro, A. Transposons and accessory genes drive adaptation in a clonally evolving fungal pathogen. *Nat. Commun.* **16**, 6982 (2025).
76. Manni, M., Berkeley, M. R., Seppey, M. & Zdobnov, E. M. BUSCO: assessing genomic data quality and beyond. *Curr. Protoc.* **1**, e323 (2021).
77. Maiorano, A., Blandino, M., Reyneri, A. & Vanara, F. Effects of maize residues on the *Fusarium* spp. infection and deoxynivalenol (DON) contamination of wheat grain. *Crop Prot.* **27**, 182–188 (2008).
78. McMullen, M., Jones, R. & Gallenberg, D. Scab of wheat and barley: a re-emerging disease of devastating impact. *Plant Dis.* **81**, 1340–1348 (1997).
79. McMullen, M., Bergstrom, G., De Wolf, E., Dill-Macky, R., Hershman, D., Shaner, G. & Van Sanford, D. A unified effort to fight an enemy of wheat and barley: *Fusarium* head blight. *Plant Dis.* **96**, 1712–1728 (2012).
80. Mikheenko, A., Prjibelski, A., Saveliev, V., Antipov, D. & Gurevich, A. Versatile genome assembly evaluation with QUAST-LG. *Bioinformatics* **34**, i142–i150 (2018).
81. Minh, B. Q., Schmidt, H. A., Chernomor, O., Schrempf, D., Woodhams, M. D., Von Haeseler, A. & Lanfear, R. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Mol. Biol. Evol.* **37**, 1530–1534 (2020).
82. Moparthi, S., Burrows, M., Mgbechi-Ezeri, J. & Agindotan, B. *Fusarium* spp. associated with root rot of pulse crops and their cross-pathogenicity to cereal crops in Montana. *Plant Dis.* **105**, 548–557 (2021).
83. Mueller, B., Groves, C. L. & Smith, D. L. Chemotype and aggressiveness evaluation of *Fusarium graminearum* and *Fusarium culmorum* isolates from wheat fields in Wisconsin. *Plant Dis.* **105**, 3686–3693 (2021).
84. Muszewska, A., Steczkiewicz, K., Stepniewska-Dziubinska, M. & Ginalski, K. Cut-and-paste transposons in fungi with diverse lifestyles. *Genome Biol. Evol.* **9**, 3463–3477 (2017).
85. Nguyen, L. T., Schmidt, H. A., Von Haeseler, A. & Minh, B. Q. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol. Biol. Evol.* **32**, 268–274 (2015).
86. O'Donnell, K., Whitaker, B. K., Laraba, I., Proctor, R. H., Brown, D. W., Broders, K., Kim, H. S., McCormick, S. P., Busman, M., Aoki, T. & Torres-Cruz, T. J. DNA

- sequence-based identification of *Fusarium*: a work in progress. *Plant Dis.* **106**, 1597–1609 (2022).
87. Okello, P. N. & Mathew, F. M. Cross pathogenicity studies show South Dakota isolates of *Fusarium acuminatum*, *F. equiseti*, *F. graminearum*, *F. oxysporum*, *F. proliferatum*, *F. solani*, and *F. subglutinans* from either soybean or corn are pathogenic to both crops. *Plant Health Prog.* **20**, 44–49 (2019).
 88. Okello, P. N., Petrovic, K., Singh, A. K., Kontz, B. & Mathew, F. M. Characterization of species of *Fusarium* causing root rot of soybean (*Glycine max* L.) in South Dakota, USA. *Can. J. Plant Pathol.* **42**, 560–571 (2020).
 89. Ou, S., Su, W., Liao, Y., Chougule, K., Agda, J. R. A., Hellinga, A. J., Lugo, C. S. B., Elliott, T. A., Ware, D., Peterson, T., Jiang, N., Hirsch, C. N. & Hufford, M. B. Benchmarking transposable element annotation methods for creation of a streamlined, comprehensive pipeline. *Genome Biol.* **20**, 275 (2019).
 90. Parikh, L., Kodati, S., Eskelson, M. J. & Adesemoye, A. O. Identification and pathogenicity of *Fusarium* spp. in row crops in Nebraska. *Crop Prot.* **108**, 120–127 (2018).
 91. Pertea, G. & Pertea, M. GFF utilities: GffRead and GffCompare. *F1000Research* **9**, ISCB-Comm (2020).
 92. Puri, K. D. & Zhong, S. The 3ADON population of *Fusarium graminearum* found in North Dakota is more aggressive and produces a higher level of DON than the prevalent 15ADON population in spring wheat. *Phytopathology* **100**, 1007–1014 (2010).
 93. Puri, K. D., Yan, C., Leng, Y. & Zhong, S. RNA-Seq revealed differences in transcriptomes between 3ADON and 15ADON populations of *Fusarium graminearum* in vitro and in planta. *PLoS ONE* **11**, e0163803 (2016).
 94. Rasche, H. & Hiltemann, S. Galactic Circos: user-friendly Circos plots within the Galaxy platform. *Gigascience* **9**, giaa065 (2020).
 95. Saupe, S. J. Molecular genetics of heterokaryon incompatibility in filamentous ascomycetes. *Microbiol. Mol. Biol. Rev.* **64**, 489–502 (2000).
 96. Seppey, M., Manni, M. & Zdobnov, E. M. BUSCO: assessing genome assembly and annotation completeness. *Methods Mol. Biol.* **1962**, 227–245 (2019).
 97. Smit, A. F. A., Hubley, R. & Green, P. RepeatMasker Open-4.0. <http://www.repeatmasker.org> (2013).
 98. Sneideris, D., Ivanauskas, A., Prakas, P., Butkauskas, D., Treikale, O., Kadziene, G., Rasiukeviciute, N., Kelpsiene, J. & Suproniene, S. Population structure of *Fusarium graminearum* isolated from different sources in one area over the course of three years. *Phytopathology* **110**, 1312–1318 (2020).
 99. Sperschneider, J., Gardiner, D. M., Thatcher, L. F., Lyons, R., Singh, K. B., Manners, J. M. & Taylor, J. M. Genome-wide analysis in three *Fusarium* pathogens identifies rapidly evolving chromosomes and genes associated with pathogenicity. *Genome Biol. Evol.* **7**, 1613–1627 (2015).

100. Sperschneider, J. & Dodds, P. N. EffectorP 3.0: prediction of apoplastic and cytoplasmic effectors in fungi and oomycetes. *Mol. Plant Microbe Interact.* **35**, 146–156 (2022).
101. Stanke, M., Schöffmann, O., Morgenstern, B. & Waack, S. Gene prediction in eukaryotes with a generalized hidden Markov model that uses hints from external sources. *BMC Bioinform.* **7**, 62 (2006).
102. Stanke, M., Diekhans, M., Baertsch, R. & Haussler, D. Using native and syntenically mapped cDNA alignments to improve de novo gene finding. *Bioinformatics* **24**, 637–644 (2008).
103. Son, M. R., Kang, M. J., Park, E. J., Lee, J. W. & Bae, E. K. First report of *Fusarium graminearum* causing tuber rot of *Gastrodia elata* in South Korea. *Plant Dis.* **109**, 938 (2025).
104. Talas, F. & McDonald, B. A. Genome-wide analysis of *Fusarium graminearum* field populations reveals hotspots of recombination. *BMC Genomics* **16**, 996 (2015).
105. Tamburic-Ilincic, L. The effect of *Fusarium graminearum* 3-ADON and 15-ADON isolates on *Fusarium* head blight symptoms, *Fusarium* damaged kernels and mycotoxins production, in inoculated and field grown winter wheat. *Can. J. Plant Sci.* **105**, 1–10 (2025).
106. Teufel, F., Almagro Armenteros, J. J., Johansen, A. R., Gíslason, M. H., Pihl, S. I., Tsirigos, K. D., Winther, O., Brunak, S., von Heijne, G. & Nielsen, H. SignalP 6.0 predicts all five types of signal peptides using protein language models. *Nat. Biotechnol.* **40**, 1023–1025 (2022).
107. Tiwari, R. K., Kumar, R., Sharma, S., Sagar, V., Aggarwal, R., Naga, K. C., Lal, M. K., Chourasia, K. N., Kumar, D. & Kumar, M. Potato dry rot disease: current status, pathogenomics and management. *3 Biotech* **10**, 503 (2020).
108. Torres-Cruz, T. J., Whitaker, B. K., Proctor, R. H., Broders, K., Laraba, I., Kim, H. S., Brown, D. W., O'Donnell, K., Estrada-Rodríguez, T. L., Lee, Y. H. & Cheong, K. FUSARIUM-ID v. 3.0: an updated, downloadable resource for *Fusarium* species identification. *Plant Dis.* **106**, 1610–1616 (2022).
109. Urban, M., Cuzick, A., Seager, J., Nonavinakere, N., Sahoo, J., Sahu, P., Iyer, V. L., Khamari, L., Martinez, M. C. & Hammond-Kosack, K. E. PHI-base - the multi-species pathogen-host interaction database in 2025. *Nucleic Acids Res.* **53**, D826–D838 (2025).
110. Vaser, R., Sović, I., Nagarajan, N. & Šikić, M. Fast and accurate de novo genome assembly from long uncorrected reads. *Genome Res.* **27**, 737–746 (2017).
111. Vogelgsang, S., Beyer, M., Pasquali, M., Jenny, E., Musa, T., Bucheli, T. D., Wettstein, F. E. & Forrer, H.-R. An eight-year survey of wheat shows distinctive effects of cropping factors on different *Fusarium* species and associated mycotoxins. *Eur. J. Agron.* **105**, 62–79 (2019).

112. von der Ohe, C., Gauthier, V., Tamburic-Iilincic, L., Brule-Babel, A., Fernando, W. D., Clear, R., Ward, T. J. & Miedaner, T. A comparison of aggressiveness and deoxynivalenol production between Canadian *Fusarium graminearum* isolates with 3-acetyl and 15-acetyldeoxynivalenol chemotypes in field-grown spring wheat. *Eur. J. Plant Pathol.* **127**, 407–417 (2010).
113. Wang, D., Zhang, Y., Zhang, Z., Zhu, J. & Yu, J. KaKs_Calculator 2.0: a toolkit incorporating gamma-series methods and sliding window strategies. *Genomics Proteomics Bioinform.* **8**, 77–80 (2010).
114. Wang, Y. B., Kang, N. H., Yang, X. Z., Ouyang, D. M., Wu, H., Chen, J. Y., Xiang, M. L. & Chen, M. First report of fruit rot caused by *Fusarium graminearum* on Ponkan (*Citrus reticulata* cv. Ponkan) in China. *Plant Dis.* **107**, 2532 (2023).
115. Wang, Q., Jiang, C., Wang, C., Chen, C., Xu, J. R. & Liu, H. Characterization of the two-speed subgenomes of *Fusarium graminearum* reveals the fast-speed subgenome specialized for adaption and infection. *Front. Plant Sci.* **8**, 140 (2017).
116. Wang, L. G., Lam, T. T. Y., Xu, S., Dai, Z., Zhou, L., Feng, T., Guo, P., Dunn, C. W., Jones, B. R., Bradley, T., Zhu, H., Guan, Y., Jiang, Y. & Yu, G. treeio: an R package for phylogenetic tree input and output with richly annotated and associated data. *Mol. Biol. Evol.* **37**, 599–603 (2020).
117. Waterhouse, A.M., Procter, J.B., Martin, D.M., Clamp, M. and Barton, G.J., 2009. Jalview Version 2—a multiple sequence alignment editor and analysis workbench. *Bioinformatics*, 25(9), pp.1189-1191.
118. Wickham, H. Getting started with ggplot2. In *ggplot2: Elegant Graphics for Data Analysis* 11–31 (Springer International Publishing, 2016).
119. Yang, Z. & Nielsen, R. Estimating synonymous and nonsynonymous substitution rates under realistic evolutionary models. *Mol. Biol. Evol.* **17**, 32–43 (2000).
120. Yulfo-Soto, G. E., Smith, H., Szarka, D., Dixon, E., Vaillancourt, L. J. & Gauthier, N. First report of *Fusarium graminearum* causing flower blight on hemp (*Cannabis sativa*) in Kentucky. *Plant Dis.* **106**, 334 (2022).
121. Zheng, X., Levine, D., Shen, J., Gogarten, S. M., Laurie, C. & Weir, B. S. A high-performance computing toolset for relatedness and principal component analysis of SNP data. *Bioinformatics* **28**, 3326–3328 (2012).
122. Zheng, X., Gogarten, S. M., Lawrence, M., Stilp, A., Conomos, M. P., Weir, B. S., Laurie, C. & Levine, D. SeqArray—a storage-efficient high-performance data format for WGS variant calls. *Bioinformatics* **33**, 2251–2257 (2017).
123. Zheng, J., Ge, Q., Yan, Y., Zhang, X., Huang, L. & Yin, Y. dbCAN3: automated carbohydrate-active enzyme and substrate annotation. *Nucleic Acids Res.* **51**, 115–121 (2023).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryGenomicDifferencesMycotoxinProfilesandCrossInfectivityPatterns.xlsx](#)