Trichothecene Genotype Profiling of Wheat 
*Fusarium graminearum* Species Complex in Paraguay

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**Abstract:** Paraguay is a non-traditional wheat-producing country in one of the warmest regions in South America. *Fusarium Head Blight* (FHB) is a critical disease affecting this crop, caused by the *Fusarium graminearum* species complex (FGSC). A variety of these species produce trichothecenes, including deoxynivalenol (DON) and its acetylated forms (3-ADON and 15-ADON) or nivalenol (NIV). This study characterized the phylogenetic relationships, and chemotype diversity of 28 strains within FGSC collected from wheat fields across different country regions. Phylogenetic analysis based on the sequence of elongation factor-1α gene (*EF-1α*) from 28 strains revealed the presence of four species in the FGSC: *F. graminearum sensu stricto*, *F. asiaticum*, *F. meridionale* and *F. cortaderiae*. Ten strains selected for further analysis revealed that all *F. graminearum* strains were 15-ADON chemotype, while the two strains of *F. meridionale* and one strain of *F. asiaticum* were NIV chemotype. Thus, the 15-ADON chemotype of *F. graminearum sensu stricto* was predominant within the *Fusarium* strains isolated in the country. This work is the first report of phylogenetic relationships and chemotype diversity among *Fusarium* strains which will help understand the population diversity of this pathogen in Paraguay.

**Keywords:** cereal; food safety; *Triticum aestivum*; mycotoxins

**Key Contribution:** This is the first report on *Fusarium* species and genotypes in wheat fields in Paraguay.

1. **Introduction**

Paraguay is one of the few countries that produce wheat in one of the world’s warmest regions and export this cereal regularly [1]. In 2019, Paraguay produced 1.1 million tons...
of wheat on 430,000 hectares and exported over 460,000 tons of grain to Brazil and other countries [1]. Unfortunately, wheat production in Paraguay is often threatened by several biotic and abiotic factors. Among the biotic limiting factors, FHB is one of Paraguay’s most important fungal diseases affecting wheat production [2]. FHB is caused by members of the Fusarium graminearum species complex (FGSC) [3], which can produce toxins for human and animal health and affect several sectors of the wheat supply chain [4]. The fungal species in the FGSC causing FHB produce trichothecenes that are strong phytotoxins and act as virulence factors to facilitate tissue colonization on sensitive host plants [5,6].

Currently, 16 monophyletic species have been identified within the FGSC [7] comprising, F. austroamericanum (lineage 1), F. meridionale (lineage 2), F. boothii (lineage 3), F. mesoamericanum (lineage 4), F. acacia-mearnsii (lineage 5), F. asiaticum, F. graminearum sensu stricto (lineage 7), F. cortaderiae (lineage 8), and eight additional monotypic lineages, including F. brasilicum, F. vorosii, F. gerlachii, F. aethiopicum, F. ussurianum, F. nepalense, F. louisianense, and US Gulf Coast population of F. graminearum [7–13].

Each Fusarium species has a specific profile of toxic secondary metabolites and a distinction can be made between chemotype I strains producing deoxynivalenol (DON) and (or) its acetylated derivatives and chemotype II strains producing nivalenol (NIV) and (or) 4-acetyl nivalenol (4-ANIV). In addition, within the DON chemotypes, chemotype IA producing 3-ADON and chemotype IB producing 15-ADON were distinguished [14,15].

The species F. graminearum sensu stricto and F. asiaticum produce 3-ADON, 15-ADON, and NIV. All other species of the FGSC produce mainly NIV and (or) 3-ADON as well as some trichothecene variant forms (15-acetyldeoxyvalenol, 3-acetyldeoxynivalenol, and nivalenol), which are species-specific and associated with pathogenicity [17]. Trichothecene gene cluster has been recognized as a 26-kb segment of DNA constituted of three loci in F. graminearum and F. sporotrichioides: a single-gene TRI101 locus, a two-gene TRI1-TRI16 locus, and a twelve-gene locus. These genes function in the biosynthesis, regulation, and transport of the mycotoxins across the plasma membrane [18,19]. TRI13 and TRI7 are the most important genes to determine the production of DON or NIV genotype [20].

There is a large diversity in the FHB causing species in the Southern Cone region of South America. For example, the Argentina F. graminearum populations from wheat are genotypically diverse and belong to F. graminearum sensu stricto; populations from Argentina were like those populations from Brazil with the same haplotype [21,22]. High genotypic diversity with significant differences within subpopulations and high gene flow would indicate that isolated strains from Argentina are part of the same unique and widespread population [23,24].

While F. meridionale, F. asiaticum, F. graminearum, F. cortaderiae, and F. austroamericanum have been reported to be associated with FHB in Brazil [22,25–27], F. graminearum sensu stricto was the most frequently isolated species in Uruguay [22,28].

Argentina, Brazil, and Uruguay represent the largest wheat-producing region in the Southern Cone, where F. graminearum genotypes producing 15-ADON are predominant [27]. However, genotypes producing NIV, 3-ADON have also been reported [21,25–27,29–34]. To date, there is no genotypic information on Fusarium species isolates from Paraguay. Since the 1970s, no epidemics of Fusarium wilt have been documented in Paraguay, and there are no data on the toxin production potential of the species present in the fields. In 1972 and 1975, two FHB and foliar blights epidemics caused wheat farmers to lose as much as 70% of their production, resulting in severe economic losses [35]. Previous studies have reported F. graminearum as the prevalent fungus producing DON mycotoxin in Paraguay [2,36–39].

Given the lack of data on genotypes and their association with the potential trichothecene production in Paraguay, this study aimed to determine the species composition and trichothecene genotypes of a set of strains collected from wheat fields.
2. Results

2.1. Identification of Fusarium Strains to Species Level

Genetic polymorphisms in the DNA sequence of EF-1α were analyzed for 28 Fusarium strains isolated in this study to identify their species [8]. Based on the phylogenetic analysis of the EF-1α gene, 23 strains were grouped as *F. graminearum sensu stricto* and confirmed by the presence of the reference strain NRRL66037 [40]. In addition, a single strain AWPYCT087 belonged to *F. cortaderiae* together with the reference strain NRRL 29,297 [41]. The remaining three strains formed a monophyletic clade with *F. meridionale* reference strain NRRL 28,436 [3], and one strain AWPY177 belonged to *F. asiaticum* together with the reference strain NRRL6101 [3] (Figure 1).

Figure 1. Maximum likelihood phylogeny of *Fusarium graminearum* Species Complex based on the alignment of EF-1α gene. Bootstrap values (percentage, based on 1000 replications) are shown on branches.
The reference sequence *Fusarium* spp. was downloaded from the National Center for Biotechnology Information. *F. pseudograminearum* and *F. culmorum* were used as outgroups. *Strains from Paraguay.*

2.2. Trichothecene Genotypes

Trichothecene genotypes of 10 selected strains were determined based on PCR amplification of *TRI3* and *TRI12* genes [42]. All *F. graminearum* strains, eight in our study, were 15-ADON chemotype. Otherwise, the two *F. meridionale* strains and the *F. asiaticum* strain were NIV chemotypes. These results indicate that all isolates studied have the potential capability to produce trichothecene mycotoxin.

3. Discussion

This work is the first phylogenetic and molecular analysis to determine the trichothecene genotype of FHB strains prevalent in FGSC populations collected from wheat fields in Paraguay.

The species belonging to FGSC are known to possess three specific profiles of trichothecene production, including nivalenol (NIV) and its acetyl derivatives, DON, and primarily 3-acetyldeoxynivalenol, 3-ADON and DON and primarily 15-ADON [43,44].

Based on the *EF-1α* gene sequences of wheat isolates, we were able to identify four species within FGSC. Of the examined strains, 82% belonged to *F. graminearum* s.s., 11% to *F. meridionale*, 3% to *F. asiaticum* and 3% to *F. cortaderiae*. Concerning the trichothecene genotypes, all *F. graminearum* s.s. strains have a 15-ADON genotype, and the two strains of *F. meridionale* and one strain of *F. asiaticum* exhibit the NIV genotype.

Several studies have been carried out to identify FGSC genotypes associated with FHB in wheat, barley, rice, and maize in the Southern Cone Region, including Argentina, Brazil, Uruguay, and Paraguay (Table 1).

| Country          | Matrix         | Presence | Prevalence | Year | Reference |
|------------------|----------------|----------|------------|------|-----------|
| Argentina        | Wheat          | +        | +          | +    | +         | 2011      | [32]         |
| Argentina        | Wheat          | +        |            | +    | +         | 2017      | [29]         |
| Argentina        | Wheat          | +        |            | +    | +         | 2014      | [45]         |
| Argentina        | Durum wheat    | +        |            | +    | +         | 2017      | [30]         |
| Uruguay          | Wheat          | +        | +          | +    | +         | 2013      | [31]         |
| Uruguay          | Wheat          | +        | +          | +    | +         | 2013      | [28]         |
| Uruguay          | Wheat          | +        | +          | +    | +         | 2013      | [33]         |
| Brazil           | Wheat          | +        | +          | +    | +         | 2012      | [25]         |
| Brazil           | Barley         | +        | +          | +    | +         | 2011      | [34]         |
| Brazil           | Wheat and Barley| +       | +          | +    | +         | 2020      | [46]         |
| Paraguay         | Wheat          | +        | +          |      |           |           | This study   |

(*+*) indicates the presence and prevalence of a particular trichothecene genotype.

The results of the present study support findings from Argentina, where all 112 *F. graminearum* s.s isolates obtained from 28 locations belonged to the 15-ADON chemotype [45]. However, they coincide better with a three-year Brazilian study conducted on the barley fields in the State of Rio Grande do Sul. In this study, the authors reported
the presence of three species within the FGSC. All 15-ADON strains were identified as *F. graminearum s.s.*, while all NIV strains were identified as *F. meridionale* [22,34], similar to our findings.

In another extensive study carried out in barley and wheat fields in the State of Paraná, Brazil, the authors determined that within the FGSC, *F. graminearum s.s.* with 15-ADON genotype was dominant (63%), followed by *F. meridionale* NIV genotype (23.1%), *F. cortaderiae* NIV (7%) or 3-ADON (2.6%) genotypes, and *F. austroamericanum* (3.8%) 3-ADON genotype [46].

Similarly, *F. graminearum s.s.* was the most frequently isolated species (97%) in Uruguay, although *F. cortaderiae* and *F. austroamericanum* were also identified [21]. The authors also reported 15-ADON (95%) as the predominant chemotype, followed by 3-ADON (3%) and NIV (2%). Most *F. graminearum s.s.* showed 15-ADON chemotype, and *F. austroamericanum* and *F. cortaderiae* isolates were 3-ADON and NIV chemotypes, respectively. Another four-year study from Uruguay confirmed that all the *F. graminearum* isolates presented the 15-ADON genotype. While *F. cortaderiae*, *F. asiaticum* and *F. brasiliicum* isolates shared the NIV type, the single *F. austroamericanum* isolate had the 3-ADON type [28].

Therefore, the species composition of FGSC in the Southern Cone region (Argentina, Brazil, Uruguay, and Paraguay) is predominated by the presence of *F. graminearum* isolates with 15-ADON genotype. Other species, such as *F. meridionale*, *F. asiaticum* and *F. cortaderiae*, are of NIV genotype, and *F. austroamericanum* of 3-ADON genotype [43]. These results coincide with reports from North America, central Europe, southern Russia, and some parts of Asia where the 15-ADON genotype dominates but different from northern Europe, where 3-ADON dominates [5].

The continuous survey and surveillance of genotypes and chemotypes of the FHB pathogens are critical to determine the risk of pathogenic and toxigenic genotypes [47] and improve the FHB management strategies, especially under a climate change scenario [30]. To explain genotype distribution in different geographic regions, hypotheses based on grain/seed shipment, international trade, long-distance spore transportation, and environmental conditions have been proposed [5].

It must be emphasized that a variable FGSC population with different genotypes can adapt to different ecological environments, such as the hosts, temperature regime, crop rotation schedule, and agronomic management. Although 3-ADON-producing strains have been reported to be more aggressive than the 15-ADON population in susceptible wheat, the former isolates exhibited a greater capacity for DON production than the 15-ADON isolates. Given the toxicological differences between DON and NIV, it is crucial to monitor the population of genotypes and to determine the chemotypes of strains present in any geographical region [5]. Even so, in wheat, DON is an important virulence factor, and strains that produce DON are more virulent than those that do not. However, other specific aspects of the pathogen related to virulence must be considered [48,49]. In strains of different chemotypes and genotypes, comparable accumulation of both acetyl derivatives could be because acetyl derivatives biosynthesis is regulated by temperature [50].

The need to carry out long-term studies with a larger number of fungal strains from the wheat-producing region of Paraguay is well understood. In addition, the analysis of the distribution of FGSC and trichothecene genotypes in the cereal crops will help to understand the relationship between disease and mycotoxin contamination observed in the country. Such studies will also help develop effective management strategies to control the disease and mycotoxin contamination [51]. Our results place Paraguay at par with other countries in the region, mainly because of the fungal populations’ toxicogenic potential.

To our knowledge, this is the first study that used *Fusarium* strains from Paraguay to analyze the phylogenetic relationships and genotype diversity. Therefore, this study helps to understand the population diversity of FGSC in the country.
4. Conclusions
Phylogenetic analysis of 28 strains revealed the presence of four species within FGSC in wheat cultivated in Paraguay: *F. graminearum* s.s., *F. asiaticum*, *F. meridionale* and *F. cortaderiae*. Of the ten selected strains for trichothecene genotype analysis, all *F. graminearum* s.s. strains belonged to the 15-ADON genotype, the two *F. meridionale* strains and *F. asiaticum* strain presented the NIV genotype. As a result, the 15-ADON genotype of *F. graminearum* sens s.s. is predominantly found within the *Fusarium* strains isolated from wheat.

This work is the first study to use *Fusarium* strains from Paraguay to analyze the phylogenetic relationships and chemotype diversity. We believe it essential to understand the population composition of FGSC in the country.

5. Materials and Methods
5.1. Fungal Isolates
Based on the morphological characters of the fungi, 28 isolates (strains) were identified as belonging to the FGSC and confirmed by sequencing translation elongation factor-1 alpha (*EF-1α*) [8]. The strains were collected from commercial wheat fields in four locations: Itapúa, Alto Paraná, Caazapá, and Canindeyú, representing major production regions of Paraguay. These were preserved as spore suspensions in 15% glycerol frozen at −80 °C [27] in the Collection of Cultures of Microorganisms of the National University of Asunción (CCM-UNA).

5.2. DNA Isolation
DNA was extracted with cetyltrimethylammonium bromide (CTAB) method from cultures grown in PDA for one week at 22 ± 1 °C. The resulting mycelia were harvested by plates’ surface scraping and stored frozen at −20 °C until ground and extracted with CTAB [32]. The DNA obtained was quantified using a DS-11-DeNovix spectrophotometer and was stored at −25 °C in a freezer.

5.3. Phylogenetic Analysis
For phylogenetic analysis, *EF-1α* gene was amplified and sequenced using EF-1 and EF-2 primers (Table 2). Agarose gels of 0.8% concentration were made in 0.5× TBE (Tris borate EDTA) Buffer and run at 90 V, 100 mA for 60 min. Amplification products were compared to a 100 bp molecular weight marker, EZ Load™ 100bp Molecular Ruler (#170-8352) [52]). For gel staining, the 1× Diamond red intercalator, Diamond™ Nucleic Acid Dye from Promega, was used [53]. The gels obtained were visualized using a Gel Documentation System, UV light in Gel Doc EZ–Biorrad [54]. The amplified products were sequenced by the automatic pyro-sequencing method, Sanger dideoxy sequencing method, in Macrogen, Korea [55]. *EF-1α* DNA sequences of 28 strains, together with known NRRL strains of FGSC from the GenBank, were aligned using ClustalW algorithm of MEGA 7.0.26 software [56]. The phylogenetic tree was constructed using the Maximum Likelihood method with 1000 bootstrap replicates. The best-fit model of molecular evolution was selected based on Bayesian Information Criterion scores [57].
Table 2. Primers used in this study.

| Primer Name | Gene | Sequence 5′–3′ | Reference |
|-------------|------|---------------|-----------|
| 3CON        | TRI3 | TGGCAAGAAGCTGGTTCAC | [44] |
| 3NA         | TRI3 | GTGCACAGAATAATACGAGC | [44] |
| 3D15A       | TRI3 | ACTGACCCAAGCTGGCCTTC | [44] |
| 3D3A        | TRI3 | CGCAATGGCTAACAACATG | [44] |
| 12CON       | TRI12| CATGACATGGTGATGTC | [44] |
| 12NF        | TRI12| TCTCTCCTGTGTTATCTTG | [44] |
| 12-15F      | TRI12| TACACGTGTCGAACCTTC | [44] |
| 12-3F       | TRI12| TCTTGCCAAGCCCGTGCA | [44] |
| EF-1        | EF-1 | ATGGTAAGGARGACAAGAC | [3,8] |
| EF-2        | EF-1 | GGARGTACCAGTSATCATGGT | [3,8] |

5.4. Trichothecene Genotype Determination

The primers used for PCR-based identification of mycotoxin biosynthetic genes are presented in Table 2. The trichothecene genotypes of the strains were determined in a PCR reaction with primers for TRI3 and TRI12 alleles [42,58].

PCR reactions included 20 ng of genomic DNA as a template in a 50 µL reaction composed of 1× reaction buffer, 2 mM MgCl₂, 1.25 U Taq DNA polymerase (Promega), 0.2 mM dNTPs, and 2 µM of each primer. PCR was performed in a thermal cycler (BioRad) with an initial denaturation step at 95 °C for 2 min; 30 cycles of 94 °C (1 min), 55 °C (1 min), and 72 °C (3 min); and a final extension step at 72 °C for 10 min [58]. PCR products were separated by electrophoresis on 2% agarose gels. Gels were stained, photographed, and further analyzed [32].

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References
1. Cámera Paraguaya de Exportadores y Comercializadores de Cereales y Oleaginosas Área de Siembra, Producción y Rendimiento – Capeco. Available online: https://capeco.org.py/area-de-siembra-produccion-y-rendimiento/ (accessed on 8 August 2021).
2. Arrúa Alvarenga, A.A. El Presente y El Futuro de La Investigación de Fusariosis de La Espiga. In Sexto Seminario Nacional del Trigo, del Grano al Pan; Kohli, M., Cubilla, L., Cabrera, G., Eds.; Cámera Paraguaya de Exportadores y Comercializadores de Cereales y Oleaginosas, Instituto de Biotecnología Agrícola: Asunción, 2018; pp. 189–198. ISBN 9789995384975. [CrossRef] [PubMed]
3. O’Donnell, K.; Kistler, H.C.; Tacke, B.K.; Casper, H.H. Gene Genealogies Reveal Global Phylogeographic Structure and Reproductive Isolation among Lineages of Fusarium Graminearum, the Fungus Causing Wheat Scab. Proc. Natl. Acad. Sci. USA 2000, 97, 7905–7910. [CrossRef] [PubMed]
4. Wilson, W.; Dahl, B.; Nganje, W. Economic Costs of Fusarium Head Blight, Scab and Deoxynivalenol. World Mycotoxin J. 2018, 11, 291–302. [CrossRef]
5. Wang, J.; Zhao, Z.; Yang, X.; Yang, J.; Gong, A.; Zhang, J.; Chen, L.; Zhou, C. Fusarium Graminearum Species Complex and Trichothecene Genotype. In Mycotoxins and Food Safety; Sabuncuoglu, S., Ed.; IntechOpen: London, UK, 2020; p. 180. [CrossRef]
6. Yu, D.; Wang, J.; Tang, Y.; Hu, D.; Wu, A. Origin of Mycotoxin-Producing Fungal Species. Food Saf. Mycotoxins 2019, 103–112. [CrossRef]
7. Amarasinghe, C.; Sharanowski, B.; Fernando, W.G.D. Molecular Phylogenetic Relationships, Trichothecene Chemotype Diversity and Aggressiveness of Strains in a Global Collection of Fusarium Graminearum Species. Toxins 2019, 11, 263. [CrossRef] [PubMed]
8. O’Donnell, K.; Ward, T.J.; Geiser, D.M.; Corby Kistler, H.; Aoki, T. Genealogical Concordance between the Mating Type Locus and Seven Other Nuclear Genes Supports Formal Recognition of Nine Phylogenetically Distinct Species within the Fusarium Graminearum Clade. Fungal Genet. Biol. 2004, 41, 600–623. [CrossRef] [PubMed]
9. Starkey, D.E.; Ward, T.J.; Aoki, T.; Gale, L.R.; Kistler, H.C.; Donnell, K.O.; Geiser, D.M.; Suga, H. Global Molecular Surveillance Reveals Novel Fusarium Head Blight Species and Trichothecene Toxin Diversity. Fungal Genet. Biol. 2007, 44, 1191–1204. [CrossRef]
10. Yli-Mattila, T.; Gagkaeva, T.; Ward, T.J.; Aoki, T.; Kistler, H.C.; O’Donnell, K. A Novel Asian Clade within the Fusarium Graminearum Species Complex Includes a Newly Discovered Cereal Head Blight Pathogen from the Russian Far East. Mycologia 2009, 101, 841–852. [CrossRef]
11. Gale, L.R.; Harrison, S.A.; Ward, T.J.; O’Donnell, K.; Milus, E.A.; Gale, S.W.; Kistler, H.C. Nivalenol-Type Populations of Fusarium Graminearum and F. Asianicum Are Prevalent on Wheat in Southern Louisiana. Phytopathology 2011, 101, 124–134. [CrossRef]
12. Sarver, B.A.J.; Ward, T.J.; Gale, L.R.; Broz, K.; Corby Kistler, H.; Aoki, T.; Nicholson, P.; Carter, J.; O’Donnell, K. Novel Fusarium Head Blight Pathogens from Nepal and Louisiana Revealed by Multilocus Genealogical Concordance. Fungal Genet. Biol. 2011, 48, 1096–1107. [CrossRef]
13. Wang, J.; Ndoye, M.; Zhang, J.; Li, H.; Liao, Y. Population Structure and Genetic Diversity of the Fusarium Graminearum Species Complex. Toxins 2011, 3, 1020–1037. [CrossRef] [PubMed]
14. Mielniczuk, E.; Skwarylo-Bednarz, B. Fusarium Head Blight, Mycotoxins and Strategies for Their Reduction. Agronomy 2020, 10, 509. [CrossRef]
15. Miller, J.D.; Greenhalgh, R.; Wang, Y.; Lu, M. Trichothecene Chemotypes of Three Fusarium Species. Mycologia 1991, 83, 121–130. [CrossRef]
16. Aoki, T.; Ward, T.J.; Kistler, H.C.; O’Donnell, K. Systematics, Phylogeny and Trichothecene Mycotoxin Potential of Fusarium Head Blight Cereal Pathogens. Mycologia 2012, 62, 91–102. [CrossRef]
17. Desjardins, A.E. Fusarium Mycotoxins. Chemistry, Genetics, and Biology; American Phytopathological Society: St. Paul, MN, USA, 2006; ISBN 978-0-89054-335-1. [CrossRef]
18. Proctor, R.H.; McCormick, S.P.; Alexander, N.J.; Desjardins, A.E. Evidence That a Secondary Metabolic Biosynthetic Gene Cluster Has Grown by Gene Relocation during Evolution of the Filamentous Fungus Fusarium. Mol. Microbiol. 2009, 74, 1128–1142. [CrossRef] [PubMed]
19. Villafana, R.; Ramdass, A.; Rampersad, S. Selection of Fusarium Trichothecene Toxin Genes for Molecular Detection Depends on TRI Gene Cluster Organization and Gene Function. Toxins 2019, 11, 36. [CrossRef] [PubMed]
20. Krnjaja, V.; Stanković, S.; Obradović, A.; Petrović, T.; Mandić, V.; Bijelić, Z.; Božić, M. Trichothecene Genotypes of Fusarium Graminearum Populations Isolated from Winter Wheat Crops in Serbia. Toxins 2018, 10, 460. [CrossRef]
21. Reynoso, M.M.; Ramirez, M.L.; Farnochi, M.C.; Torres, A.M.; Chulze, S.N. Population Structure of Fusarium Graminearum Species Complex Genotypes and Chemotypes in Relation to Trichothecenes Production. In Fusarium Head Blight in Latin America; Alconada Magliano, T.M., Chulze, S.N., Eds.; Springer Netherlands: Dordrecht, The Netherlands, 2013; pp. 3–13. ISBN 978-94-007-0790-4. [CrossRef]
22. Del Ponte, E.M.; Tessmann, D.J.; Spolti, P.; Kuhнем, P.R.; da Silva, C.N. Species Identification, Genetic Diversity and Phenotypic Variation Studies on the Fusarium Graminearum Complex Populations from Brazil. In Fusarium Head Blight in Latin America; Alconada, T., Chulze, S., Eds.; Springer Netherlands: Dordrecht, The Netherlands, 2013; pp. 15–29. ISBN 9789400770911. [CrossRef]
23. Yerkovich, N.; Fumero, M.V.; Cantoro, R.; Palazzini, J.M.; Chulze, S.N. Population Structure and Genetic Diversity of Fusarium Graminearum SensuStricto, the Main Wheat Pathogen Producing Fusarium Head Blight in Argentina. Eur. J. Plant Pathol. 2020, 156, 653–646. [CrossRef]
24. Con solo, V.F.; Ortega, L.M.; Salerno, G.; Astoreca, A.L.; Alconada, T.M. Genetic Diversity of Fusarium Graminearum Sensu Lato Isolates from Wheat Associated with Fusarium Head Blight in Diverse Geographic Locations of Argentina. Rev. Argent. Microbiol. 2015, 47, 245–250. [CrossRef]
25. Astolfi, P.; Reynoso, M.M.; Ramirez, M.L.; Chulze, S.N.; Alves, T.C.A.; Tessmann, D.J.; Del Ponte, E.M. Genetic Population Structure and Trichothecene Genotypes of Fusarium Graminearum Isolated from Wheat in Southern Brazil. Plant Pathol. 2012, 61, 289–295. [CrossRef]
26. Scoz, L.B.; Astolfi, P.; Rearthes, D.S.; Schmale III, D.G.; Moraeas, M.G.; Del Ponte, E.M. Trichothecene Mycotoxin Genotypes of Fusarium Graminearum SensuStricto and Fusarium Meridionale in Wheat from Southern Brazil. Plant Pathol. 2009, 58, 344–351. [CrossRef]
27. de Arruda, M.H.M.; Zchosnki, F.L.; Silva, Y.K.; de Lima, D.L.; Tessmann, D.J.; Da-Silva, P.R. Genetic Diversity of Fusarium Meridionale, F. Austroamericanum, and F. Graminearum Isolates Associated with Fusarium Head Blight of Wheat in Brazil. Trop. Plant Pathol. 2021, 46, 98–108. [CrossRef]
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28. Umpiérez-Failache, M.; Garmendia, G.; Pereyra, S.; Rodríguez-Haralambides, A.; Ward, T.J.; Vero, S. Regional Differences in Species Composition and Toxigenic Potential among Fusarium Head Blight Isolates from Uruguay Indicate a Risk of Nivalenol Contamination in New Wheat Production Areas. *Int. J. Food Microbiol.* 2013, 166, 135–140. [CrossRef]

29. Yerkovich, N.; Palazzini, J.M.; Sulyok, M.; Chulze, S.N. Trichothecene Genotypes, Chemotypes and Zearalenone Production by Fusarium Graminearum Species Complex Strains Causing Fusarium Head Blight in Argentina during an Epidemic and Non-Epidemic Season. *Trop. Plant Pathol.* 2017, 42, 190–196. [CrossRef]

30. Palacios, S.A.; Erazo, J.G.; Ciasca, B.; Lattanzio, V.M.T.; Reynoso, M.M.; Farnoči, M.C.; Torres, A.M. Occurrence of Deoxynivalenol and Deoxynivalenol-3-Glucoisole in Durum Wheat from Argentina. *Food Chem.* 2017, 230, 728–734. [CrossRef] [PubMed]

31. Pan, D.; Calero, N.; Mionetto, A.; Bettucci, L. Trichothecene Genotypes of Fusarium Graminearum from Wheat in Uruguay. *Int. J. Food Microbiol.* 2015, 162, 120–123. [CrossRef] [PubMed]

32. Reynoso, M.M.; Ramirez, M.I.; Chulze, S.N. Trichothecene Genotypes and Chemotypes in Fusarium Graminearum Strains Isolated from Wheat in Argentina. *Int. J. Food Microbiol.* 2011, 145, 444–448. [CrossRef]

33. Garmendia, G.; Pattarino, L.; Negrón, C.; Martínez-Silveira, A.; Pereyra, S.; Ward, T.J.; Vero, S. Species Composition, Toxigenic Potential and Aggressiveness of Fusarium Head Blight of Barley in Uruguay. *Food Microbiol.* 2018, 76, 426–433. [CrossRef] [PubMed]

34. Astolfi, P.; dos Santos, J.; Schneider, L.; Gomes, L.B.; Silva, C.N.; Tessmann, D.J.; Del Ponte, E.M. Molecular Survey of Trichothecene Genotypes of Fusarium Graminearum Species Complex from Barley in Southern Brazil. *Int. J. Food Microbiol.* 2011, 148, 197–201. [CrossRef]

35. Quintana de Viedma, L. Importancia y Distribución de La Fusariosis Del Trigo En El Paraguay. In *Taller sobre la fusariosis de la espiga en América del Sur*, Kohli, M.M., Ed.; Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT): Encarnación, Paraguay, 1987; pp. 39–48. ISBN 968-6172-37-2.

36. Quintana de Viedma, L.; Morel, W. Especies de Fusarium Que Afectan a Semillas de Trigo En Paraguay. In *Proceedings of the XIX Seminario Panamericano de semillas. Conferencias y resúmenes de trabajos presentados*; Cristaldo, D., Ed.; Federación Latinoamericana de Asociaciones de Semillas (FELAS); Asociación de Productores de Semillas del Paraguay (APROSEMP); Ministerio de Agricultura y Ganadería, Dirección de Semillas: Asunción, Paraguay, 2004; p. 328.

37. Quintana de Viedma, L. Toxinas de Fusarium En Semilla de Trigo En El Paraguay. In *Proceedings of the XIX Seminario Panamericano de semillas. Conferencias y resúmenes de trabajos presentados*; Cristaldo, D., Ed.; Federación Latinoamericana de Asociaciones de Semillas (FELAS); Asociación de Productores de Semillas del Paraguay (APROSEMP); Ministerio de Agricultura y Ganadería, Dirección de Semillas: Asunción, Paraguay, 2004; p. 335.

38. Arrúa Alvarenga, A.A.; Moura Mendes, J.; Carolina Cazal Martínez, C.; Eduardo Dujak Riquelme, C.; Fernández Rios, D.; María Oviedo de Cristaldo, R.; Mohan Kohli, M. Incidencia de Hongos Del Complejo Fusarium Graminearum y Acumulación de Deoxinivalenol En Líneas de Trigo. *Investig. Agrar.* 2014, 16, 43–48.

39. Arrúa Alvarenga, A.A. Avances En La Investigación de La Fusariosis de La Espiga En Paraguay. In *Quinto Seminario Nacional de Trigo: ‘Del grano al pan’; Kohli, M.M., Cubilla, L.E.; Cabrera, G., Eds.; Cámara Paraguaya de Exportadores y Comercializadores de Cereales y Oleaginosas, Instituto de Biotecnología Agrícola: Asunción, Paraguay, 2015; pp. 139–150. ISBN 978-99953-849-6-8.

40. Kelly, A.; Proctor, R.H.; Belzile, F.; Chulze, S.N.; Clear, R.M.; Cowger, C.; Elmer, W.; Lee, T.; Obanor, F.; Waalwijk, C.; et al. The Geographic Distribution and Complex Evolutionary History of the NX-2 Trichothecene Chemotype from Fusarium Graminearum. *Fungal Genet. Biol.* 2016, 95, 39–48. [CrossRef]

41. Laraiba, I.; McCormick, S.P.; Vaughan, M.M.; Geiser, D.M.; O’Donnell, K. Phylogenetic Diversity, Trichothecene Potential, and Pathogenicity within Fusarium Sambucinum Species Complex. *PloS ONE* 2021, 16, e0245037. [CrossRef]

42. Ward, T.J.; Clear, R.M.; Rooney, A.P.; O’Donnell, K.; Gaba, D.; Patrick, S.; Starkey, D.E.; Gilbert, J.; Geiser, D.M.; Nowicki, T.W. An Adaptive Evolutionary Shift in Fusarium Head Blight Pathogen Populations Is Driving the Rapid Spread of More Toxigenic Fusarium Graminearum in North America. *Fungal Genet. Biol.* 2008, 45, 473–484. [CrossRef]

43. Castañares, E.; Dinolfo, M.I.; Del Ponte, E.M.; Pan, D.; Stenglein, S.A. Species Composition and Genetic Structure of Fusarium Graminearum Species Complex Populations Affecting the Main Barley Growing Regions of South America. *Plant Pathol.* 2016, 65, 930–939. [CrossRef]

44. Desjardins, A.E. Natural Product Chemistry Meets Genetics: When Is a Genotype a Chemotype? *J. Agric. Food Chem.* 2008, 56, 7587–7592. [CrossRef]

45. Malbrán, I.; Mourellos, C.A.; Girotti, J.R.; Balatti, P.A.; Lori, G.A. Toxigenic Capacity and Trichothecene Production by Fusarium Graminearum Isolates from Argentina and Their Relationship with aggressiveness and Fungal Expansion in the Wheat Spike. *Phytopathology* 2014, 104, 357–364. [CrossRef]

46. Bertuzzi Pereira, C.; Ward, T.J.; Del Ponte, E.M.; Mara Moreira, G.; Busman, M.; McCormick, S.P.; Feksa, H.R.; De Almeida, J.L.; Tessmann, D.J. Five-Year Survey Uncovers Extensive Diversity and Temporal Fluctuations among Fusarium Head Blight Pathogens of Wheat and Barley in Brazil. *Plant Pathol.* 2020, 70, 426–435. [CrossRef]

47. Qiu, J.B.; Sun, J.T.; Yu, M.Z.; Xu, J.H.; Shi, J.R. Temporal Dynamics, Population Characterization and Mycotoxins Accumulation of Fusarium Graminearum in Eastern China. *Sci. Rep.* 2016, 6, 1–11. [CrossRef]

48. Leslie, J.F.; Moretti, A.; Mesterházy, Á.; Ameyar, K.; Singh, P.K.; Richard-Forget, F.; Chulze, S.N.; Ponte, E.M.D.; Chala, A.; et al. Key Global Actions for Mycotoxin Management in Wheat and Other Small Grains. *Toxins* 2021, 13, 725. [CrossRef]
49. Proctor, R.H.; Desjardins, A.E.; McCormick, S.P.; Plattner, R.D.; Alexander, N.J.; Brown, D.W. Genetic Analysis of the Role of Trichothecene and Fumonisins Mycotoxins in the Virulence of Fusarium. *Eur. J. Plant Pathol.* **2002**, *108*, 691–698. [CrossRef]

50. Ramírez Albuquerque, D.; Patriarca, A.; Fernández Pinto, V. Can Discrepancies between Fusarium Graminearum Trichothecene Genotype and Chemotype Be Explained by the Influence of Temperature in the Relative Production of 3-ADON and 15-ADON? *Fungal Biol.* **2021**, *125*, 153–159. [CrossRef]

51. Ji, F.; He, D.; Olaniran, A.O.; Mokoena, M.P.; Xu, J.; Shi, J. Occurrence, Toxicity, Production and Detection of Fusarium Mycotoxin: A Review. *Food Prod. Process. Nutr.* **2019**, *1*, 6. [CrossRef]

52. Bio-Rad Laboratories EZ Load 100 Bp Molecular Ruler. Available online: https://www.bio-rad.com/es-py/sku/1708352-ez-load-100-bp-molecular-ruler?ID=1708352&WT_mc_id=220128033438&WT_srch=1&WT_knsh_id=_kenshoo_clickid_&gclid=CjwKCAjw_tWRBhAwEiwALxPfofCsQnJGzZG2QBNVj7tsC8eYlUy1E_1sDInAWuVGzDI1wGDlfZlthoCrskQAVd_BwE (accessed on 19 March 2022).

53. Promega DiamondTM Nucleic Acid Dye. Available online: https://worldwide.promega.com/products/biochemicals-and-labware/biochemical-buffers-and-reagents/diamond-nucleic-acid-dye/?catNum=H1181 (accessed on 19 March 2022).

54. Bio-Rad Gel Doc EZ Gel Documentation System. Available online: https://www.bio-rad.com/es-py/product/gel-doc-ez-gel-documentation-system?ID=O4950KKG4 (accessed on 20 March 2022).

55. Humanizing Genomics Macrogen Macrogen. Available online: https://www.macrogen.com/en/main (accessed on 20 March 2022).

56. Kumar, S.; Stecher, G.; Tamura, K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol. Biol. Evol.* **2016**, *33*, 1870–1874. [CrossRef] [PubMed]

57. Crouch, J.A.; Clarke, B.B.; Hillman, B.I. Unraveling Evolutionary Relationships Among the Divergent Lineages of Colletotrichum Causing Anthracnose Disease in Turfgrass and Corn. *Phytopathology* **2006**, *96*, 46–60. [CrossRef] [PubMed]

58. Barros, G.; Zanon, M.S.A.; Abod, A.; Oviedo, M.S.; Ramírez, 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* **2012**, *29*, 293–303. [CrossRef]