Fusarium Species Associated with Maize Leaf Blight in Heilongjiang Province, China

Xi Xu 1, Li Zhang 1, Xilang Yang 1, Guijin Shen 1, Shuo Wang 1, Haolin Teng 1, Chunbo Yang 1, Xueyan Liu 1, Xiangjing Wang 1,2, Junwei Zhao 1,2,* and Wensheng Xiang 1,2,*

1 Key Laboratory of Agricultural Microbiology of Heilongjiang Province, Northeast Agricultural University, No. 600 Changjiang Road, Xiangfang District, Harbin 150030, China
2 State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100097, China

* Correspondence: zhaojunwei@neau.edu.cn (J.Z.); xiangwensheng@neau.edu.cn (W.X.)

Abstract: Fusarium spp. are among the most important plant pathogens in the world. A survey on maize leaf blight was carried out in Heilongjiang province from 2019 to 2021. Based on morphological characteristics and a phylogenetic analysis on translation elongation factor (tef1) and second-largest subunit of RNA polymerase II (rpb2) genes, 146 Fusarium isolates were obtained and grouped into 14 Fusarium species, including F. ipomoeae (20.5%), F. compactum (17.1%), F. sporotrichioides (9.59%), F. graminearum (9.59%), F. citri (8.9%), F. asiaticum (6.85%), F. verticillioides (6.85%), F. acuminatum (5.48%), F. glycines (5.48%), F. temperatum (2.74%), F. armeniacum (2.74%), Fusarium sp. (2.05%), F. flagelliforme (1.4%), and F. annulatum (0.68%). The Fusarium incarnatum-equiseti species complex (FIESC, including F. ipomoeae, F. compactum, F. citri, and F. flagelliforme) was the most prevalent, indicating an evolving occurrence of the Fusarium species causing maize leaf blight. The typical symptoms observed on the maize leaves were oval to long strip lesions, with a gray to dark gray or brownish red coloration in the center and a chlorotic area at the edges. Based on the tef1 gene, seven haplotypes of FIESC were identified in Heilongjiang province, suggesting a population expansion. This is the first report of F. ipomoeae, F. compactum, F. flagelliforme, F. citri, F. sporotrichioides, F. graminearum, F. asiaticum, F. acuminatum, F. glycines, F. temperatum, F. armeniacum, Fusarium sp., and F. annulatum causing maize leaf blight in Heilongjiang province, China. The current research is informative for managing disease, exploring the phylogenetic relationship among Fusarium species, and clarifying the diversity of Fusarium species associated with maize leaf blight.

Keywords: Fusarium spp.; maize; haplotype analysis; genetic diversity

1. Introduction

Fusarium spp. can cause several diseases in maize, such as Fusarium ear rot [1–3], Fusarium stalk rot and root rot [2,4], seedling blight [5], and maize leaf blight [6]. Regarding maize leaf blight, Fusarium verticillioides was the first pathogen, reported in 1968 [6], to cause the disease, and the only reported one up to now. However, the pathogenicity and diversity of Fusarium spp. causing maize leaf blight are still unclarified. Maize leaf blight is characterized by symptoms of irregular or spindle lesions, with gray to reddish brown coloration in the lesions’ center surrounded by a chlorotic halo. Sometimes, this disease is misjudged as northern corn leaf spot due to the similar symptoms in the field. Thus, the identification of the pathogens based only on disease symptoms in the field is difficult.

To our knowledge, the genus Fusarium includes more than 300 phylogenetic species [7] and is one of the most important plant pathogens in the world [8]. Most species within the genus can produce a diverse range of mycotoxins, causing varying degrees of acute or chronic toxic effects [1]. Therefore, the accurate identification of these mycotoxin producers is a considerable endeavor [9]. For the identification of fungi and the investigation of molecular ecology, the internal transcribed spacer (ITS) is the most sequenced DNA region [10].
However, the ITS region cannot distinguish the species complex of *Fusarium* due to its conservation [11]. By contrast, the *tef1* gene can be used to discriminate *Fusarium* species at the species or subspecies level [11,12], and the *rpb2* gene is also more informative and frequently employed, so it has been recommended that they are sequenced for *Fusarium* species identification. However, although the partial beta-tubulin gene has been used to identify several *Fusarium* species, it was not universally informative within *Fusarium* [13].

The members of *Fusarium incarnatum-equiseti* species complex (FIESC) are considered important plant pathogens. FIESC is rarely considered the major pathogen of disease epidemics, but it has been identified as a co-occurring fungal pathogen during an infection [14]. Thirty phylogenetic species within the FIESC (FIESC 1 through FIESC 30) were recognized through Multi-locus Sequence Typing (MLST) [15,16], and the species containing multiple haplotypes are designated by the addition of a lowercase letter to the phylogenetic species designation [9].

Phylogenetic and genetic diversity analyses based on multiple sequences can reveal evolutionary relationships associated with geographical regions [9]. High genetic diversity indicates greater adaptability to changing environmental conditions. In some complex evolutionary scenarios, appropriate and sufficient information may not be obtained from phylogenetic trees [17,18]. By comparison, haplotype networks can be employed to analyze the intraspecific diversity of populations, genetic processes, and the biogeography and history of populations [18,19].

To date, there has been little research on pathogenicity, genetic diversity, and the haplotype groups of pathogenic *Fusarium* species isolated from symptomatic maize leaves in China. Hence, the purposes of the present study were to: (i) describe the morphological characterization and phylogenetic relationships based on *tef1* and *rpb2* genes of *Fusarium* species responsible for maize leaf blight in Heilongjiang province, (ii) evaluate the pathogenicity of different *Fusarium* species, and (iii) determine the haplotype diversity of FIESC based on *tef1* associated with maize leaf blight.

2. Materials and Methods

2.1. Fusarium Isolates Collection

From 2019 to 2021, a total of 132 symptomatic maize leaves were collected from 10 different maize-growing counties or cities in Heilongjiang province. The symptomatic maize leaves were cut with a sterilized scalpel, superficially disinfected with a 2% solution of sodium hypochlorite for 1 min and 75% ethanol for 30 s, rinsed thrice with sterile distilled water, and air-dried on sterile filter papers under aseptic conditions. Pure cultures were obtained by single-spore isolation and maintained on PDA (potato dextrose agar) at 25 °C for 7 days. *Fusarium* isolates were obtained and preserved on PDA slants at 4 °C and 20% glycerol at −80 °C for temporary storage and long-term storage, respectively.

2.2. Morphological Characterization

All *Fusarium* isolates were incubated on PDA plate in the dark at 25 °C for 7 days. Colony color and colony texture were observed for each isolate. To determine the size of well-developed macroconidia (*n = 30*) and the number of septa, these *Fusarium* isolates were incubated on PDA plates at 25°C for 7 days with light/dark cycle of 8/16 h. The macroconidia were observed under light microscopy (Zeiss Axiolab5 equipped with an Axiocam 208 color industrial digital camera).

2.3. DNA Extraction and Sequence Analysis

Fresh mycelia were harvested from cultures grown on PDA supplemented with streptomycin (50 mg/L) and tetracycline (50 mg/L) for 7 days at 28 °C. The extraction of fungal genomic DNA was performed as Ramdial et al. described [9]. The sequences of the translation elongation factor 1-alpha (*tef1*) gene, second-largest subunit of RNA polymerase II gene (*rpb2*), and partial beta-tubulin gene were amplified by the primers EF-1/EF-2, RPB2-5f2/RPB2-7cr, and Bt2a/Bt2b [13,20], respectively. The PCR products
were sent to Jilin Comate Bioscience Co. Ltd. for purification and sequencing. Sequences of 146 Fusarium isolates were searched against GenBank and FUSARIOID-ID database (www.fusarium.org, accessed date: 1 September 2022) [21] by Basic Local Alignment Search Tool (BLAST) analysis and then deposited into the NCBI GenBank (Table 1).

Table 1. List of GenBank accession numbers of Fusarium isolates obtained from symptomatic maize leaves collected from Heilongjiang province and reference strains used in this study.

| Isolates.   | Latitude and Longitude | Species         | GenBank Accession Nos. |
|-------------|------------------------|-----------------|------------------------|
|             |                        |                 | tef1       | rpb2       | Beta-Tubulin |
| HA-z142     | 126.738196, 45.753014  | *F. ipomoeae*   | OM985077  | OP436018   | OP642121    |
| HA-z11      | 126.738196, 45.753014  | *F. ipomoeae*   | OM985078  | OP436019   | OP642120    |
| HA-z12      | 126.738196, 45.753014  | *F. ipomoeae*   | OM985079  | OP436020   | OP642119    |
| HA-z13      | 126.738196, 45.753014  | *F. ipomoeae*   | OM985080  | OP436021   | OP642118    |
| HA-z14      | 126.738196, 45.753014  | *F. ipomoeae*   | OM985081  | OP436022   | OP642117    |
| HA-z15      | 126.738196, 45.753014  | *F. ipomoeae*   | OM985082  | OP436023   | OP642116    |
| HA-z16      | 126.738196, 45.753014  | *F. ipomoeae*   | OM985083  | OP436024   | OP642115    |
| HA-z17      | 126.738196, 45.753014  | *F. ipomoeae*   | OM985084  | OP436025   | OP642114    |
| HA-z18      | 126.738196, 45.753014  | *F. ipomoeae*   | OM985085  | OP436026   | OP642113    |
| HA-z19      | 126.738196, 45.753014  | *F. ipomoeae*   | OM985086  | OP436027   | OP642112    |
| HA-z20      | 126.738196, 45.753014  | *F. ipomoeae*   | OM985087  | OP436028   | OP642111    |
| HA-z21      | 126.738196, 45.753014  | *F. ipomoeae*   | OM985088  | OP436029   | OP642110    |
| HA-z22      | 126.738196, 45.753014  | *F. ipomoeae*   | OM985089  | OP436030   | OP642109    |
| HA-x22      | 126.868024, 45.850128  | *F. ipomoeae*   | OM985106  | OP436031   | OP642108    |
| HA-xy82     | 126.933932, 45.769353  | *F. ipomoeae*   | OM985109  | OP436032   | OP642122    |
| HA-xy83     | 126.933932, 45.769353  | *F. ipomoeae*   | OM985110  | OP436033   | OP642123    |
| HA-31       | 126.868024, 45.850128  | *F. ipomoeae*   | OM985118  | OP436034   | OP642107    |
| SH-11       | 127.270457, 46.64457   | *F. ipomoeae*   | OM985119  | OP436035   | OP642106    |
| SH-63       | 127.270457, 46.64457   | *F. ipomoeae*   | OM985120  | OP436036   | OP642105    |
| WC-31       | 127.22506, 44.93996    | *F. ipomoeae*   | OM985124  | OP436037   | OP642104    |
| QQ-41       | 124.340195, 47.29158   | *F. ipomoeae*   | OM985125  | OP436038   | OP642103    |
| SH-62       | 127.270457, 46.64457   | *F. ipomoeae*   | OM985126  | OP436039   | OP642124    |
| HA-x201     | 126.738196, 45.753014  | *F. ipomoeae*   | OM985127  | OP436040   | OP642125    |
| HA-21       | 126.868024, 45.850128  | *F. ipomoeae*   | OM985128  | OP436041   | OP642126    |
| HA-22       | 126.868024, 45.850128  | *F. ipomoeae*   | OM985129  | OP436042   | OP642127    |
| HA-x21      | 126.868024, 45.850128  | *F. ipomoeae*   | OM985130  | OP436043   | OP642102    |
| HA-212      | 126.868024, 45.850128  | *F. ipomoeae*   | OM985140  | OP436044   | OP642101    |
| DQ-n22      | 125.835845, 46.329205  | *F. ipomoeae*   | OM985182  | OP436045   | OP642100    |
| JX-21       | 132.477436, 46.339951   | *F. ipomoeae*   | OM985183  | OP436046   | OP642098    |
| DQ-n31      | 125.835845, 46.329205  | *F. ipomoeae*   | OM985184  | OP436047   | OP642099    |
| HA-61       | 126.868024, 45.850128  | *F. compactum*  | OM985144  | OP435951   | OP642130    |
| HA-111      | 126.868024, 45.850128  | *F. compactum*  | OM985102  | OP435952   | OP642131    |
| JX-y11      | 132.477436, 46.339951   | *F. compactum*  | OM985123  | OP435953   | OP642132    |
| HA-621      | 126.868024, 45.850128  | *F. compactum*  | OM985145  | OP435975   | OP642128    |
Table 1. Cont.

| Isolates. | Latitude and Longitude | Species       | GenBank Accession Nos. |
|-----------|------------------------|---------------|------------------------|
| SYS-31    | 132.768479, 46.215238  | *F. compactum* | tef1: OM985146         |
|           |                        |               | rpb2: OP435954         |
| HA-z152   | 126.738196, 45.753014  | *F. compactum* | Beta-Tubulin: OP642129 |
| HA-z31    | 126.738196, 45.753014  | *F. compactum* | GenBank Accession Nos.  |
| HA-z32    | 126.738196, 45.753014  | *F. compactum* | tef1: OM985147         |
|           |                        |               | rpb2: OP435955         |
| HA-z33    | 126.738196, 45.753014  | *F. compactum* | Beta-Tubulin: OP642133 |
| HA-z34    | 126.738196, 45.753014  | *F. compactum* | GenBank Accession Nos.  |
| HA-z35    | 126.738196, 45.753014  | *F. compactum* | tef1: OM985148         |
|           |                        |               | rpb2: OP435956         |
| HA-z36    | 126.738196, 45.753014  | *F. compactum* | Beta-Tubulin: OP642134 |
| HA-z37    | 126.738196, 45.753014  | *F. compactum* | GenBank Accession Nos.  |
| HA-z38    | 126.738196, 45.753014  | *F. compactum* | tef1: OM985149         |
|           |                        |               | rpb2: OP435957         |
| HA-z39    | 126.738196, 45.753014  | *F. compactum* | Beta-Tubulin: OP642135 |
| HA-z310   | 126.738196, 45.753014  | *F. compactum* | GenBank Accession Nos.  |
| HA-z311   | 126.738196, 45.753014  | *F. compactum* | tef1: OM985150         |
|           |                        |               | rpb2: OP435958         |
| HA-z312   | 126.738196, 45.753014  | *F. compactum* | Beta-Tubulin: OP642136 |
| HA-x12    | 126.868024, 45.850128  | *F. citri*    | GenBank Accession Nos.  |
| QTH-21    | 131.139405, 45.733699  | *F. citri*    | tef1: OM985160         |
| QTH-23    | 131.139405, 45.733699  | *F. citri*    | rpb2: OP435965         |
| HA-z1125  | 126.738196, 45.753014  | *F. citri*    | Beta-Tubulin: OP642146 |
| HA-z171   | 126.738196, 45.753014  | *F. citri*    | GenBank Accession Nos.  |
| HA-z172   | 126.738196, 45.753014  | *F. citri*    | tef1: OM985168         |
|           |                        |               | rpb2: OP435969         |
| HA-z173   | 126.738196, 45.753014  | *F. citri*    | Beta-Tubulin: OP642147 |
| HA-z174   | 126.738196, 45.753014  | *F. citri*    | GenBank Accession Nos.  |
| HA-z175   | 126.738196, 45.753014  | *F. citri*    | tef1: OM985162         |
|           |                        |               | rpb2: OP435970         |
| HA-z176   | 126.738196, 45.753014  | *F. citri*    | Beta-Tubulin: OP642152 |
| HA-x11    | 126.868024, 45.850128  | *F. flagelliforme* | GenBank Accession Nos.  |
| QTH-23    | 131.139405, 45.733699  | *F. flagelliforme* | tef1: OM985174         |
|           |                        |               | rpb2: OP435943         |
| HA-x11    | 126.868024, 45.850128  | *F. flagelliforme* | Beta-Tubulin: OP642161 |
| HA-x51    | 126.868024, 45.850128  | *F. graminearum* | GenBank Accession Nos.  |
| HA-a31    | 126.868024, 45.850128  | *F. graminearum* | tef1: OM985105         |
|           |                        |               | rpb2: OP435920         |
| HG-11     | 130.440826, 47.312952  | *F. graminearum* | Beta-Tubulin: OP642154 |
| QTH-23    | 131.139405, 45.733699  | *F. graminearum* | GenBank Accession Nos.  |
| SH-x72    | 127.270457, 46.64457   | *F. graminearum* | tef1: OM985108         |
|           |                        |               | rpb2: OP435983         |
|           |                        |               | Beta-Tubulin: OP642203 |
| Isolates. | Latitude and Longitude | Species | GenBank Accession Nos. | tef1 | rpb2 | Beta-Tubulin |
|-----------|------------------------|---------|------------------------|------|------|-------------|
| SYS-y21   | 132.768479, 46.215238  | F. graminearum | OM985111 OP435984 OP642204 |
| SYS-21    | 132.768479, 46.215238  | F. graminearum | OM985199 OP435985 OP642205 |
| SYS-141   | 132.768479, 46.215238  | F. graminearum | OM985200 OP435986 OP642206 |
| SYS-142   | 132.768479, 46.215238  | F. graminearum | OM985201 OP435987 OP642207 |
| SYS-143   | 132.768479, 46.215238  | F. graminearum | OM985202 OP435988 OP642208 |
| SYS-144   | 132.768479, 46.215238  | F. graminearum | OM985203 OP435989 OP642209 |
| SYS-145   | 132.768479, 46.215238  | F. graminearum | OM985204 OP435990 OP642210 |
| SYS-146   | 132.768479, 46.215238  | F. graminearum | OM985205 OP435991 OP642211 |
| SYS-147   | 132.768479, 46.215238  | F. graminearum | OM985206 OP435992 OP642212 |
| HA-a142   | 126.868024, 45.850128  | F. graminearum | OM985138 OP435993 OP642213 |
| SYS-x71   | 131.583118, 46.462499  | F. asiaticum  | OM985092 OP436053 OP642088 |
| SYS-x91   | 131.583118, 46.462499  | F. asiaticum  | OM985093 OP436054 OP642089 |
| HA-x72    | 126.868024, 45.850128  | F. asiaticum  | OM985094 OP436055 OP642090 |
| HG-x62    | 130.440826, 47.312952   | F. asiaticum  | OM985095 OP436056 OP642091 |
| SYS-x62   | 131.583118, 46.462499  | F. asiaticum  | OM985096 OP436057 OP642092 |
| SYS-x131  | 131.583118, 46.462499  | F. asiaticum  | OM985097 OP436058 OP642093 |
| SYS-x132  | 131.583118, 46.462499  | F. asiaticum  | OM985098 OP436059 OP642094 |
| SYS-x133  | 131.583118, 46.462499  | F. asiaticum  | OM985099 OP436060 OP642095 |
| SYS-x134  | 131.583118, 46.462499  | F. asiaticum  | OM985100 OP436061 OP642096 |
| SYS-x135  | 131.583118, 46.462499  | F. asiaticum  | OM985101 OP436062 OP642097 |
| HA-zh142  | 126.738196, 45.753014  | F. temperatum | OM985107 OP436049 OP642174 |
| QTH-X332  | 131.139405, 45.733699  | F. temperatum | OM985131 OP436050 OP642171 |
| QTH-X331  | 131.139405, 45.733699  | F. temperatum | OM985132 OP436051 OP642173 |
| QTH-X33   | 131.139405, 45.733699  | F. temperatum | OM985133 OP436052 OP642172 |
| HA-z113   | 126.738196, 45.753014  | Fusarium sp.  | OM985112 OP436063 OP642168 |
| HA-b113   | 126.738196, 45.753014  | Fusarium sp.  | OM985113 OP436064 OP642170 |
| HA-Z1131  | 126.738196, 45.753014  | Fusarium sp.  | OM985143 OP436065 OP642169 |
| SYS-x11   | 131.583118, 46.462499  | F. sporotrichioides | OM985209 OP436017 OP642176 |
| SYS-x61   | 131.583118, 46.462499  | F. sporotrichioides | OM985210 OP436016 OP642177 |
| SYS-x1    | 131.583118, 46.462499  | F. sporotrichioides | OM985211 OP436015 OP642178 |
| SYS-x2    | 131.583118, 46.462499  | F. sporotrichioides | OM985212 OP436014 OP642179 |
| HG-12     | 130.440826, 47.312952   | F. sporotrichioides | OM985213 OP436013 OP642180 |
| SYS-33    | 132.768479, 46.215238  | F. sporotrichioides | OM985214 OP436012 OP642181 |
| SYS-101   | 132.768479, 46.215238  | F. sporotrichioides | OM985215 OP436011 OP642182 |
| SYS-102   | 132.768479, 46.215238  | F. sporotrichioides | OM985216 OP436010 OP642183 |
| SYS-103   | 132.768479, 46.215238  | F. sporotrichioides | OM985217 OP436009 OP642184 |
| SYS-104   | 132.768479, 46.215238  | F. sporotrichioides | OM985218 OP436008 OP642185 |
| SYS-105   | 132.768479, 46.215238  | F. sporotrichioides | OM985219 OP436007 OP642186 |
| SYS-51    | 132.768479, 46.215238  | F. sporotrichioides | OM985220 OP436006 OP642187 |
| HG-y102   | 130.440826, 47.312952   | F. sporotrichioides | OM985121 OP436005 OP642188 |
Table 1. Cont.

| Isolates   | Latitude and Longitude   | Species       | GenBank Accession Nos. |
|------------|--------------------------|---------------|------------------------|
|            |                          | tef1 | rpb2 | Beta-Tubulin |
| HG-DBy101  | 130.440826, 47.312952    | F. sporotrichioides | OM985122 | OP436004 | OP642189 |
| SH-z61     | 127.270457, 46.64457     | F. acuminatum  | OM985115 | OP435923 | OP642072 |
| SH-61      | 127.270457, 46.64457     | F. acuminatum  | OM985116 | OP435922 | OP642073 |
| SH-41      | 127.270457, 46.64457     | F. acuminatum  | OM985117 | OP435924 | OP642074 |
| HA-a72     | 126.868024, 45.850128    | F. acuminatum  | OM985221 | OP435925 | OP642075 |
| HA-a161    | 126.868024, 45.850128    | F. acuminatum  | OM985222 | OP435926 | OP642076 |
| HA-a162    | 126.868024, 45.850128    | F. acuminatum  | OM985223 | OP435927 | OP642077 |
| HA-a163    | 126.868024, 45.850128    | F. acuminatum  | OM985224 | OP435928 | OP642078 |
| HA-a164    | 126.868024, 45.850128    | F. acuminatum  | OM985225 | OP435929 | OP642079 |
| HA-a1211   | 126.868024, 45.850128    | F. armeniacum  | OM985134 | OP435979 | OP642214 |
| HA-13      | 126.868024, 45.850128    | F. armeniacum  | OM985135 | OP435978 | OP642215 |
| HA-a121    | 126.868024, 45.850128    | F. armeniacum  | OM985136 | OP435976 | OP642216 |
| HA-a122    | 126.868024, 45.850128    | F. armeniacum  | OM985137 | OP435977 | OP642217 |
| HL-42      | 132.943466, 45.768947    | F. verticillioides | OM985139 | OP435994 | OP642190 |
| DQ-n32     | 125.835845, 46.329205    | F. verticillioides | OM985141 | OP435995 | OP642191 |
| SH-n12     | 127.270457, 46.64457     | F. verticillioides | OM985142 | OP435996 | OP642192 |
| JX-123     | 132.477436, 46.339951    | F. verticillioides | OM985181 | OP435997 | OP642193 |
| SH-n11     | 127.270457, 46.64457     | F. verticillioides | OM985187 | OP435998 | OP642194 |
| SH-n201    | 127.270457, 46.64457     | F. verticillioides | OM985188 | OP435999 | OP642195 |
| SH-n202    | 127.270457, 46.64457     | F. verticillioides | OM985189 | OP436000 | OP642197 |
| SH-n203    | 127.270457, 46.64457     | F. verticillioides | OM985190 | OP436001 | OP642197 |
| SH-n204    | 127.270457, 46.64457     | F. verticillioides | OM985191 | OP436002 | OP642199 |
| SH-n205    | 127.270457, 46.64457     | F. verticillioides | OM985192 | OP436003 | OP642199 |
| JX-3352    | 132.477436, 46.339951    | F. glycines    | OM985193 | OP435937 | OP642080 |
| JX-335     | 132.477436, 46.339951    | F. glycines    | OM985194 | OP435930 | OP642081 |
| HA-171     | 126.868024, 45.850128    | F. glycines    | OM985195 | OP435936 | OP642082 |
| HA-172     | 126.868024, 45.850128    | F. glycines    | OM985196 | OP435935 | OP642083 |
| HA-173     | 126.868024, 45.850128    | F. glycines    | OM985197 | OP435934 | OP642084 |
| HA-174     | 126.868024, 45.850128    | F. glycines    | OM985198 | OP435933 | OP642085 |
| WC-b53     | 127.22506, 44.93996      | F. glycines    | OM985208 | OP435932 | OP642086 |
| HA-z1412   | 126.738196, 45.753014    | F. glycines    | OM985180 | OP435931 | OP642087 |
| WC-22      | 127.22506, 44.93996      | F. annulatum   | OM985207 | OP436048 | OP642175 |
| NRRL 34034 | -                        | F. ipomoeae    | GQ505636 | GQ505814 | -        |
| LC0455     | -                        | F. ipomoeae    | MK289580 | MK289734 | -        |
| NRRL 45996 | -                        | F. ipomoeae    | GQ505671 | GQ505849 | -        |
| CBS 140909 | -                        | F. ipomoeae    | MN170479 | MN170412 | -        |
| NRRL 28029 | -                        | F. compactum   | GQ505602 | GQ505780 | -        |
| NRRL 36318 | -                        | F. compactum   | GQ505646 | GQ505824 | -        |
| NRRL 6548  | -                        | F. flagelliforme | GQ505589 | GQ505767 | -        |
| CBS 731.87 | -                        | F. flagelliforme | GQ505600 | GQ505778 | -        |
Table 1. Cont.

| Isolates       | Latitude and Longitude | Species         | GenBank Accession Nos. |
|----------------|------------------------|-----------------|------------------------|
|                |                        |                 | tef1                   | rpb2                   | Beta-Tubulin |
| LC12147        | -                      | F. arcuatisporum | MK289584               | MK289739               | -            |
| NRRL 32997     | -                      | F. arcuatisporum | GQ505624               | GQ505802               | -            |
| NRRL 45997     | -                      | F. clavus        | GQ505672               | GQ505850               | -            |
| NRRL 34037     | -                      | F. clavus        | GQ505638               | GQ505638               | -            |
| LC7937         | -                      | F. citri         | MK289640               | GQ505816               | -            |
| LC7922         | -                      | F. citri         | MK289634               | MK289788               | -            |
| NRRL 66939     | -                      | Fusarium sp.     | MW233217               | MW233561               | -            |
| FRC R-9121     | -                      | Fusarium sp.     | MW233213               | MW233557               | -            |
| CBS 462.94     | -                      | F. sporotrichioides | MN120771               | MN120750               | -            |
| NRRL 53430     | -                      | F. sibiricum     | HM744684               | MW233474               | -            |
| NRRL 6227      | -                      | F. armeniacum    | HM744692               | JX171560               | -            |
| FRC R-09335    | -                      | F. armeniacum    | GQ915501               | GQ915485               | -            |
| NRRL 13818     | -                      | F. asiaticum     | AF212451               | MW233412               | -            |
| NRRL 46738     | -                      | F. asiaticum     | FJ240299               | -                      | -            |
| NL19-100008    | -                      | F. graminearum   | MZ921906               | MZ921775               | -            |
| CBS 136009     | -                      | F. graminearum   | MW928838               | MW928826               | -            |
| NRRL 54216     | -                      | F. acuminatum    | HM068314               | HM068334               | -            |
| JW 289003      | -                      | F. acuminatum    | MZ921908               | MZ921777               | -            |
| CBS 130180     | -                      | F. verticillioides | MW402024               | MW402740               | -            |
| CBS 131389     | -                      | F. verticillioides | MN534047               | MN534288               | -            |
| CBS 135541     | -                      | F. temperatum    | MW402051               | KU604284               | -            |
| CBS 130323     | -                      | Fusarium sp.     | MH485018               | MH484927               | -            |
| CBS 214.49     | -                      | F. glycines      | MH484960               | MH484869               | -            |
| CBS 127316     | -                      | F. annulatum     | MW402021               | MW402738               | -            |
| CBS 100001     | -                      | Macroconia       | KM231959               | HQ728164               | -            |

Bold accession numbers were generated from other studies.

2.4. Phylogenetic Relationships among Fusarium Isolates

The rpb2 (794–896 bp), tef1 (546–686 bp), and β-tubulin (332–356 bp) gene sequences of Fusarium isolates were also compared to the sequences available in the FUSARIOID-ID database (www.fusarium.org, accessed date: 1 September 2022) to collect related sequences for inclusion in phylogenetic analysis. Multiple sequence alignments were correspondingly inferred in Molecular Evolutionary Genetics Analysis (MEGA) 7 software [22] using the MUSCLE (multiple sequence comparison by log-expectation) program [23] and refined manually if necessary. To generate concatenated datasets, single gene sequences (tef1 and rpb2) were manually combined utilizing BioEdit [24]. Phylogenetic tree based on the concatenated sequences of tef1 and rpb2 genes was built using the maximum likelihood (ML) method in MEGA 7, respectively. ML tree was generated from bootstrapping 1000 replicates. Bootstrap values ≥ 70% were shown in phylogenetic trees. The sequences from the Fusarium spp. type strains, initially identified as closely related to the sequences herein, were finally included by the preliminary BLAST searches.
2.5. Pathogenicity Tests

All Fusarium isolates were used to evaluate their pathogenicity based on the method described by Xu et al. [25]. To fulfill Koch’s postulates, 10 healthy, surface-sterilized, and four to five leaf-stage maize seedlings (var. Demeiya 3) for each Fusarium isolate were inoculated with Fusarium spore suspension (1 × 10⁶ spores/mL). Twenty maize seedlings sprayed with sterile distilled water served as controls. All seedlings sealed with plastic bags were maintained in a greenhouse at 25 °C with 90% relative humidity and a light/dark cycle of 12/12 h.

Disease severity (DS) and disease incidence (DI) were assessed 14 days post-inoculation. DS was measured based on a 0–9 scale described by Rafael et al. [26] and Xu et al. [25]: 0 (no visible symptoms), 1 (0 up to 0.5%), 2 (0.5–1.6%), 3 (1.6–5.0%), 4 (5.0–15%), 5 (15–37%), 6 (37–66%), 7 (66–87%), 8 (87% to 96%), and 9 (96–100%). DI was computed by following formula: \[ DI = \frac{100 \times \sum (n \times \text{corresponding DS})}{N \times 9} \], where \( n \) is the number of infected inoculation leaves corresponding to each disease rating, and \( N \) is the total number of inoculation leaves. Disease incidence was computed by following formula: disease incidence = number of diseased leaves/total number of inoculated leaves of living maize plants. A least significant difference (LSD) test was used for statistical analysis at a significance level of \( p < 0.05 \) with the Statistical Package for Social Sciences (SPSS) software (v. 20.0; SPSS Inc., Wacker Drive, Chicago, IL, USA, Illinois IBM Corp., 2012. IBM). All re-isolated pathogens from inoculated maize leaves were identified using morphological and molecular methods mentioned above. Each experiment was repeated two times.

2.6. DNA Polymorphism

DNA Sequence Polymorphism software version 6 was used to individually determine the DNA polymorphism relative degree of the tef1 gene sequences [27]. Furthermore, Tajima’s D, Fu and Li’s D, and Fu and Li’s F were used to determine neutrality test statistics. Significant values of these tests indicate the presence of population changes [28,29]. DNA polymorphism analyses were only performed on FIESC and not on other Fusarium species on account of the limited number of isolates from those species obtained in the current study.

2.7. Haplotype Analysis

Haplotype networks were individually generated based on the tef1 gene sequences of 70 FIESC isolates (including 30 F. ipomoeae isolates, 25 F. compactum isolates, 13 F. citri isolates, and 2 F. flagelliforme isolates in the present study) using PopART v. 1.7 (Allan Wilson Centre Imaging Evolution Initiative) to evaluate genealogy patterns of the haplotypes [19]. The aligned haplotype sequences were used to construct a TCS network [30,31].

3. Results

3.1. Fungal Isolation and Morphological Characterization

In this study, 146 Fusarium isolates were obtained from symptomatic maize leaves in China (Table 1), which were initially classified into 11 groups based on their morphological features, including the Fusarium incarnatum-equiseti species complex (FIESC, including F. ipomoeae, F. compactum, F. citri, and F. flagelliforme in this study), F. sporotrichioides, F. armeniacum, F. asiaticum, F. graminearum, Fusarium sp., F. acuminatum, F. glycines, F. annulatum, F. temperatum, and F. verticillioides (Table 2).

Seventy isolates were identified as the members of FIESC and produced white to light yellow aerial mycelia. The bottom of the plate turned white to pale brown with time. The macroconidia were slightly curved at the apex with three to five septa and ranged from 39.6 to 83.5 × 3.9 to 5.2 µm (\( n = 30 \), Figures 1a–d and 2a–d) in size.
Table 2. Geographic origins and number of Fusarium isolates recovered from symptomatic maize leaves with macroscopic symptoms of leaf blight collected from 10 locations in Heilongjiang province, China.

| Geographic Origins | Number of Fusarium Isolates |
|--------------------|-----------------------------|
|                    | FIESC | F. sporotrichoides | F. armeniacum | F. asiaticum | F. graminearum sp. | F. acuminatum | F. glycines | F. annulatum | F. temperatum | F. verticillioides |
| Daqing city        | 2     | 0                  | 0             | 0            | 0               | 0            | 0          | 0           | 0            | 0               |
| Harbin city        | 56    | 4                  | 1             | 2            | 3               | 5            | 5          | 0           | 1            | 0               |
| Hegang city        | 0     | 3                  | 0             | 1            | 1               | 0            | 0          | 0           | 0            | 0               |
| Jixi city          | 5     | 0                  | 0             | 0            | 0               | 0            | 0          | 0           | 0            | 0               |
| Qiqihar city       | 1     | 0                  | 0             | 0            | 0               | 0            | 0          | 0           | 0            | 0               |
| Qitaihe city       | 1     | 0                  | 0             | 1            | 0               | 0            | 0          | 0           | 0            | 0               |
| Shuangyashan city  | 1     | 11                 | 0             | 8            | 9               | 0            | 0          | 0           | 0            | 0               |
| Suihua city        | 3     | 0                  | 0             | 1            | 3               | 1            | 0          | 0           | 0            | 0               |
| Hulin country      | 0     | 0                  | 0             | 0            | 0               | 0            | 0          | 0           | 0            | 0               |
| Wuchang city       | 1     | 0                  | 0             | 0            | 0               | 0            | 1          | 0           | 0            | 0               |
| Total              | 70    | 14                 | 4             | 10           | 14              | 3            | 8          | 8           | 10           | 6.85            |

Percentage * 47.95 9.59 2.74 6.85 9.59 2.05 5.48 5.48 0.68 2.74 6.85

* Percentage = n/N \times 100%, where n is the number of isolates for one species of Fusarium, and N is the total number of isolates for all Fusarium species.

Figure 1. Macroconidia or microconidia of representative isolates of 14 Fusarium species. (a) F. compactum; (b) F. ipomoeae; (c) F. citri; (d) F. flagelliforme; (e) F. temperatum; (f) F. acuminatum; (g) F. armeniacum; (h) F. asiaticum; (i) F. annulatum; (j) Fusarium sp.; (k) F. graminearum; (l) F. glycines; (m) F. verticillioides; (n) F. sporotrichoides.
Their macroconidia were slender with a distinct curve of the apical cell, mostly three- to five-septate, and measured 31.3 to 65.3 × 4.0 to 6.5 μm (n = 30, Figures 1f and 2k).

Fourteen F. sporotrichioides isolates produced white-pink aerial mycelia and had dark red pigmentation. Their macroconidia were straight or slightly curved with five to seven septa and measured 25.4 to 97.7 × 3.4 to 5.8 μm (n = 30, Figures 1h and 2h).

Ten isolates producing pink to fluffy dark red aerial mycelia, and red to aubergine pigmentation with age, were classified under F. asiaticum. Their macroconidia were falcate with three to five septa, and measured 21.5 to 58.3 × 2.1 to 3.6 μm (n = 30, Figures 1i and 2m).

Figure 2. Colony appearance of representative isolates of 14 Fusarium species. (a) F. compactum; (b) F. ipomoeae; (c) F. citri; (d) F. flagelliforme; (e) F. verticillioides (f) F. sporotrichioides; (g) F. armeniacum; (h) F. asiaticum; (i) F. graminearum; (j) Fusarium sp.; (k) F. acuminatum; (l) F. glycines; (m) F. annulatum; (n) F. temperatum.

Fusarium sp. isolates produced white to yellow colonies and red pigmentation. Their macroconidia were curved with three to five septa and measured 34.0 to 71.6 × 3.2 to 4.7 μm (n = 30, Figures 1g and 2g).

The colonies of four F. armeniacum isolates were white to light pink. The macroconidia were prominently curved with three to five septa and had sizes ranging from 35.6 to 59.3 μm × 4 to 4.6 μm (n = 30, Figures 1g and 2g).

Fourteen F. graminearum isolates produced white-pink aerial mycelia and had dark red pigmentation. Their macroconidia were straight or slightly curved with five to seven septa and measured 25.4 to 97.7 × 3.4 to 5.8 μm (n = 30, Figures 1k and 2i).

Three Fusarium sp. isolates produced white to yellow colonies and red pigmentation. Their macroconidia were curved with three to five septa and measured 34.0 to 71.6 × 3.2 to 4.7 μm (n = 30, Figures 1j and 2j).

The colonies of eight F. acuminatum isolates were whitish-pink or carmine to rose red. Their macroconidia were slender with a distinct curve of the apical cell, mostly three- to five-septate, and measured 31.3 to 65.3 × 4.0 to 6.5 μm (n = 30, Figures 1f and 2k).
The colonies of eight *F. glycines* isolates produced fluffy, white aerial hyphae and a dark red pigment. Their macroconidia were three- to seven-septate, slightly curved, and ranged from 53.3 to 117.9 μm × 3.3 to 4.5 μm (n = 30, Figures 1I and 2I) in size.

The aerial mycelia of the *F. annulatum* isolates were white to cream-colored and turned violet with age, and their macroconidia were straight or slightly curved and contained three to five septa, with sizes of 21.5 to 58.3 × 2.1 to 3.6 μm (n = 30, Figures 1I and 2M).

The colonies of four *F. temperatum* isolates were pinkish-white and produced mostly three-septate macroconidia. Their macroconidia measured 34.5 to 60.8 × 3.2 to 4.1 μm (n = 30, Figures 1E and 2N).

Ten *F. verticillioides* isolates formed cottony white to greyish-purple colonies with a dark yellow to purple-gray underside. Their microconidia were abundant and mainly showed clavate shapes measuring 4.2 to 7.5 × 2.1 to 3.8 μm (n = 30, Figures 1M and 2E). However, there were no macroconidia of the *F. verticillioides* isolates observed in this study.

### 3.2. Phylogenetic Analysis

The sequences of the *tef1*, *rpb2*, and beta-tubulin genes of all the *Fusarium* isolates obtained in this study were searched against the FUSARIOID-ID database (www.fusarium.org, accessed date: 1 September 2022) using a BLAST analysis (Table S1). For further molecular verification, a multilocus phylogenetic analysis (MLSA) was further performed based on the concatenated sequences (*tef1* and *rpb2* genes) of all the *Fusarium isolates* (Figure 3). These results indicated that all the Fusarium isolates could be grouped into 14 clades, including *F. ipomoeae*, *F. compactum*, *F. sporotrichioides*, *F. citri*, *F. graminearum*, *F. asiaticum*, *F. verticillioides*, *F. acuminatum*, *F. glycines*, *F. temperatum*, *F. armeniacum*, *Fusarium sp.*, *F. flagelliforme*, and *F. annulatum*.

![Figure 3. Phylogenetic tree obtained from maximum likelihood analysis based on the concatenated sequences of *tef1* and *rpb2* genes. Support values at nodes representing RA × ML bootstrap percentages with values ≥70 are shown above the branches.](image-url)
3.3. Pathogenicity Tests

Two weeks after inoculation, the pathogenicity test revealed that all the Fusarium species could cause similar maize leaf blight symptoms (Figure 4). Small oval to fusiform or long striped spots initially appeared on the maize leaves three days post-inoculation, in which the lesions’ centers were gray to reddish brown and surrounded by a chlorotic area. The lesions gradually enlarged with time and merged into each other. In a severe case, the infected leaves were withered. The symptoms observed under greenhouse conditions were similar to the symptoms of maize leaf blight in the field (Figure 4a). No symptoms were observed in the control group. In addition, all the Fusarium species were consistently re-isolated and confirmed based on morphological and molecular methods, while no Fusarium isolates were obtained from the control group, thus fulfilling Koch’s postulates. The average disease incidence and average disease index caused by the Fusarium species ranged from 23 to 74% and from 52 to 85, respectively (Figures 5 and 6; Table S2). Moreover, all the Fusarium isolates were pathogenic towards maize leaves (var. Demeiya 3) and caused maize leaf blight in the inoculation study. In addition, F. graminearum showed the highest virulence, followed by Fusarium sp., F. glycines, F. acuminatum, F. compactum, F. temperatum, F. asiaticum, F. citri, F. verticilloides, F. armeniacum, F. ipomeae, F. annulatum, F. sporotrichioides, and F. flagelliforme.

Figure 4. (a) Leaf blight symptoms on maize leaves caused by Fusarium species in the field; (b–o) Typical symptoms observed in greenhouse on maize leaves after inoculation with: (b) F. ipomeae; (c) F. compactum; (d) F. flagelliforme; (e) F. asiaticum; (f) F. armeniacum; (g) F. citri; (h) F. sporotrichioides; (i) Fusarium sp.; (j) F. glycines; (k) F. graminearum; (l) F. annulatum; (m) F. temperatum; (n) F. verticilloides; (o) F. acuminatum.

Figure 5. Disease index for maize leaves inoculated with different Fusarium species.
3.4. Haplotype Analyses and DNA Polymorphism

The haplotype networks based on the tef1 gene sequences of 70 FIESC isolates (including 30 F. ipomoeae isolates, 25 F. compactum isolates, 2 F. flagelliforme isolates, and 13 F. citri isolates) obtained in this study were used to determine evolutionary relationships among the haplotypes. Most haplotypes within one species were closely related and separated by one to three mutations.

A total of seven haplotypes were identified: the F. ipomoeae isolates were assigned to Hap 1 and 4; F. compactum isolates were assigned to Hap 2, 5, and 6; F. flagelliforme isolates were assigned to Hap 3; and F. citri isolates were assigned to Hap 7 (Figure 7).

Meanwhile, Hap 1, 2, 4, 5, and 7 were shared haplotypes (Figure 7). Hap 1 was the most predominant haplotype, and presented in six locations (Harbin city, Wuchang city, Daqing city, Suihua city, Jixi city, and Qiqihar city). Hap 2 was found in Harbin city and Jixi city. Hap 4 was found in Harbin city and Wuchang city. Hap 5 was distributed in Harbin city and Shuangyashan city. Hap 7 was detected in Harbin city and Qitahei city. Furthermore, two private haplotypes (Hap 3 and 6) were present in Harbin city and Jixi city, respectively. However, there was no obvious center between these predominant haplotypes. In addition, a low degree of nucleotide diversity (0.02706) and a high degree of haplotype diversity (Hd) (0.778) were found. Tajima’s D, Fu and Li’s D, and Fu and Li’s F tests were negative with no significance (p > 0.10, Table S3).
Figure 7. TCS analyses and the haplotype distribution based on the tef1 gene sequences of 70 FIESC isolates obtained in this study. Each haplotype is represented by a circle, the size of which is proportional to the haplotype frequency.

4. Discussion

As far as we know, this is the first systematic study of the *Fusarium* species associated with maize leaf blight. In this study, 146 *Fusarium* isolates delimited to 14 *Fusarium* species were obtained from symptomatic maize leaves in Heilongjiang province. To analyze the genetic relationship between these *Fusarium* isolates obtained in the current study, phylogenetic trees were constructed only based on the concatenated sequences of *tef1* and *rpb2* genes because these two genes were more informative and frequently employed, while the beta-tubulin gene was not universally informative in *Fusarium* [13]. A total of 14 *Fusarium* species were identified, including *F. ipomoeae*, *F. compactum*, *F. sporotrichioides*, *F. citri*, *F. graminearum*, *F. asiaticum*, *F. verticillioides*, *F. acuminatum*, *F. glycines*, *F. temperatum*, *F. armeniacum*, *Fusarium* sp., *F. flagelliforme*, and *F. annulatum*. Except for *F. verticillioides*, which was the only reported pathogen inciting maize leaf blight [6], the remaining *Fusarium* species were all first reported in Heilongjiang province, China, suggesting that the composition of *Fusarium* species causing maize leaf blight may have changed.

Furthermore, considerable pathogenicity differences were found among the different *Fusarium* species. *F. graminearum* showed significantly greater average disease incidence and average disease indices than those of other *Fusarium* species, followed by *Fusarium* sp.,
F. glycines, F. acuminatum, F. compactum, F. temperatum, F. asiaticum, F. citri, F. verticillioides, F. armeniacum, F. ipomoeae, F. annulatum, F. sporotrichioides, and F. flagelliforme. Members of FIESC are generally considered co-occurring pathogens [32,33], and the moderate aggressiveness of FIESC in this study seems to confirm the previous conclusion. FIESC was the most predominant in this study. Members of FIESC have been frequently isolated from maize, soybean, rice, barley, wheat, and so on [34–39] and have also been reported to cause leaf blight in peanut plants [40] and Cyperus iria [41].

The haplotype groups of FIESC associated with maize leaf blight were first identified in this work. The predominant haplotype (Hap 1) represented multiple locations (Harbin city, Wuchang city, Daqing city, Suihua city, Jixi city, and Qiqihar city). It is well-known that older haplotypes may have a wider geographic distribution, which suggests that Hap 1 has lasted in the population for a long time [42]. The rest of the haplotypes may represent recently evolved lineages [4]. Furthermore, haplotypes 2, 5, and 6 belonged to the F. compactum clade; haplotypes 1 and 4 belonged to the F. ipomoeae clade; haplotype 3 belonged to the F. flagelliforme clade; and haplotype 7 belonged to the F. citri clade. These FIESC isolates were distributed in different clades in the haplotype network, which suggests that the haplotype network could effectively differentiate the *Fusarium* species complex and further confirmed our identification results. Moreover, the F. flagelliforme haplotype (Hap 3) and F. citri haplotype (Hap 7) were observed in external parts of the haplotype network and showed more mutation events from their nearest haplotypes, which indicated that these two species have an older evolutionary relationship. In addition, the high haplotype diversity and low nucleotide diversity indicated a population expansion [43].

In conclusion, the current study focused on the pathogenicity and genetic diversity of *Fusarium* species causing maize leaf blight in Heilongjiang province, China, and is the first to report F. ipomoeae, F. compactum, F. flagelliforme, F. citri, F. sporotrichioides, F. graminearum, F. asiaticum, F. verticillioides, F. acuminatum, F. glycines, F. temperatum, F. armeniacum, Fusarium sp., and F. annulatum as the causal agents. *Fusarium* can cause various maize diseases; therefore, clarifying the population composition of *Fusarium* spp. on maize leaves will provide information for the overall control of maize diseases.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jof8111170/s1, Table S1. Tef1 gene sequences similarity to reference strain; Table S2. Disease index and disease incidence on maize leaves inoculated with different Fusarium isolates; Table S3. DNA polymorphism data for FIESC isolates based on tef1 gene sequences.

**Author Contributions:** X.X., L.Z., X.Y., G.S. and S.W. performed the experiments. H.T. and C.Y. prepared the figures and tables. X.X. and X.L. analyzed the data. X.W., W.X. and J.Z. designed the experiments and reviewed the manuscript. All authors have read and agreed to the published version of the manuscript.

**Funding:** This work was supported in part by grants from the Key Program of the National Natural Science Foundation of China (No. 32030090), the Outstanding Youth Project of Natural Science Foundation of Heilongjiang Province (YQ2021C012), the Postdoctoral research fund of Heilongjiang Province (LBH-Q21072), the Academic Backbone Project of Northeast Agricultural University (20XG33), and the National Natural Youth Science Foundation of China (No. 31701858).

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Sequences have been deposited in GenBank. The data presented in this study are openly available in NCBI. Publicly available datasets were analyzed in this study. These data can be found here: https://www.ncbi.nlm.nih.gov/, accessed on 3 September 2022.

**Conflicts of Interest:** The authors declare that there are no conflict of interest.
28. Tajima, F. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* **1989**, *123*, 585–595. [CrossRef]

29. Fu, Y.X.; Li, W.H. Statistical tests of neutrality of mutations. *Genetics* **1993**, *123*, 693–709. [CrossRef]

30. Templeton, A.R.; Crandall, K.A.; Sing, C.F. A cladistics analysis of phenotypic associations with haplotypes inferred from restriction end nuclease mapping and DNA sequence data III. Cladogram estimation. *Genetics* **1992**, *132*, 619–633. [CrossRef]

31. Clement, M.; Snell, Q.; Walke, P.; Posada, D.; Crandall, K.A. TCS: Estimating gene genealogies. In Proceedings of the 16th International Parallel and Distributed Processing Symposium (IPDPS 2002), Fort Lauderdale, FL, USA, 15–19 April 2002.

32. Summerell, B.A.; Salleh, B.; Leslie, J.F. A utilitarian approach to *Fusarium* identification. *Plant Dis.* **2003**, *87*, 117–128. [CrossRef]

33. Jurado, M.; Vázquez, C.; Patiño, B.; González-Jaén, M.T. PCR detection assays for the trichothecene-producing species *Fusarium graminearum*, *Fusarium culmorum*, *Fusarium poae*, *Fusarium equiseti* and *Fusarium sporotrichioides*. *Syst. Appl. Microbiol.* **2005**, *28*, 562–568. [CrossRef]

34. Wang, M.M.; Chen, Q.; Diao, Y.Z.; Duan, W.J.; Cai, L. *Fusarium incarnatum-equiseti* complex from China. *Pers. Mol. Phylogeny Evol. Fungi* **2019**, *43*, 70–89. [CrossRef] [PubMed]

35. Marín, P.; Moretti, A.; Ritieni, A.; Jurado, M.; Vázquez, C.; González-Jaén, M.T. Phylogenetic analyses and toxigenic profiles of *Fusarium equiseti* and *Fusarium acuminatum* isolated from cereals from southern Europe. *Food Microbiol.* **2012**, *31*, 229–237. [CrossRef]

36. Barros, G.; Zanon, M.S.; Palazzini, J.M.; Haidukowski, M.; Pascale, M.; Chulze, S. Trichothecenes and zearalenone production by *Fusarium equiseti* and *Fusarium semitectum* species isolated from Argentinean soybean. *Food Addit. Contam.-Chem. Anal. Control Expo. Risk Assess.* **2012**, *29*, 1436–1442. [CrossRef] [PubMed]

37. Avila, C.F.; Moreira, G.M.; Nicolli, C.P.; Gomes, L.B.; Abreu, L.M.; Pfennig, L.H.; Haidukowski, M.; Moretti, A.; Logrieco, A.; Del Ponte, E.M. *Fusarium incarnatum-equiseti* species complex associated with Brazilian rice: Phylogeny, morphology and toxigenic potential. *Int. J. Food Microbiol.* **2019**, *306*, 108267. [CrossRef] [PubMed]

38. Piacentini, K.C.; Rocha, L.O.; Savi, G.D.; Cernielli-Queiroz, L.; De Carvalho Fontes, L.; Correa, B. Assessment of toxigenic *Fusarium* species and their mycotoxins in brewing barley grains. *Toxins* **2019**, *11*, 31. [CrossRef] [PubMed]

39. Castellá, G.; Cabañes, F.J. Phylogenetic diversity of *Fusarium incarnatum-equiseti* species complex isolated from Spanish wheat. *Antonie Van Leeuwenhoek* **2014**, *106*, 309–317. [CrossRef]

40. Thirumalaisamy, P.P.; Dutta, R.; Jadon, K.S.; Nataraja, M.V.; Padvi, R.D.; Rajayaguru, R.; Yusufzai, S. Association and characterization of the *Fusarium incarnatum-F. equiseti* species complex with leaf blight and wilt of peanut in India. *J. Gen. Plant Pathol.* **2018**, *85*, 83–89. [CrossRef]

41. Gupta, V.S.; Razdan, V.K.; John, D.T.; Sharma, B.C. First report of leaf blight of *Cyperus iria* caused by *Fusarium equiseti* in India. *Plant Dis.* **2013**, *97*, 838. [CrossRef]

42. Posada, D.; Crandall, K.A. Evaluation of methods for detecting recombination from DNA sequences: Computer simulations. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 13757–13762. [CrossRef]

43. Matić, S.; Tabone, G.; Garibaldi, A.; Gullino, M.L. Alternaria leaf spot caused by *Alternaria* species: An emerging problem on ornamental plants in Italy. *Plant Dis.* **2020**, *104*, 2275–2287. [CrossRef]