Journal of Agricultural Science and Technology (JAST), 28(4)
In Press, Pre-Proof Version

Interactions of Maize-Pathogenic *Fusarium* Species with *Macrophomina phaseolina* Shed Light on Host Preference in Crop Rotation

Ayşe Emine Görmez[1], Semiha Yüceer[2], Talap Talapov[1], and Canan Can[1*]

ABSTRACT

Survey studies during the 2021–2022 maize growing season in Türkiye's Central and Southeastern Anatolia Regions identified *Fusarium* species in seven provinces (Eskişehir, Konya, Karaman, Gaziantep, Şanlıurfa, Mardin, and Diyarbakır). Pathogenicity tests revealed 31 pathogenic isolates among 115 *Fusarium* spp., with three isolates causing the highest disease severity at a 50% infection rate. These isolates showed moderate pathogenicity in stems and weak pathogenicity in roots. For species identification, EF-1alpha and RPB regions of 28 isolates were amplified, and sequences were compared to the NCBI database via BLAST analysis. Results indicated *Fusarium proliferatum, F. subglutinans,* and *F. verticillioides* as the dominant species. Cross-pathogenicity tests with *F. proliferatum, F. subglutinans, F. verticillioides,* and *Macrophomina phaseolina*, alone and in combinations, were performed on barley, wheat, bean, corn, garlic, sorghum, and oat genotypes. All tested plants showed varying disease severity, with garlic being the most affected. *M. phaseolina* exhibited the least pathogenicity, while its combination with *F. verticillioides* caused the greatest damage. These findings are particularly significant considering the pathogens' disease impacts and mycotoxin production capacities.

Keywords: Maize, *Fusairum* spp., *Macrophomima phaseolina*, cross pathogenicity.

INTRODUCTION

Maize (*Zea mays* L.) is among the most produced grains due to its use in many areas, adaptability and productivity. According to USDA (United States Department of Agriculture) January 2024 data, 1235,73 million tons of maize were produced from 204,00 million hectares of land in the world. UK took the first place in production with of 389,69 million tons in an area of 35,01 million hectares followed by China and Brazil. Türkiye ranks twentieth with 8,20 million tons of production in an area of 0,65 million hectares.

Fusarium species are widespread globally, from tropical to temperate and harsh climates (Early, 2009). Temperature significantly influences the population dynamics and community structure

[1] Department of Biology, Faculty of Arts and Sciences, Gaziantep University, Gaziantep, Türkiye.
[2] Biological Control Research Institute, Adana, Türkiye.
*Corresponding author; e-mail: can@gantep.edu.tr

of *Fusarium* spp. (Saremi and Burgess, 2000). *Fusarium* species adapt to various habitats and contaminate crops worldwide (Ekwomadu, 2019). Most species are moisture-tolerant and cause damage even in dry soils. The disease is more prevalent in fields with continuous cereal cultivation and excessive nitrogen fertilizer use, leading to root and crown rot, reduced yield and quality, and plant lodging (Yıldırım et al., 2016).

Fusarium spp. are critical for food security, especially in cereal production. The genus includes many agriculturally significant plant pathogens, mycotoxin producers, and opportunistic human pathogens (Ma et al., 2013). *Fusarium* infections in humans and animals have also become clinically significant, with limited treatment options (Jain et al., 2011).

The *Fusarium fujikuroi* species complex contains several species contaminating maize and other cereals with fumonisins (Aoki et al., 2014). While *F. verticillioides* is a major mycotoxin producer in maize, *F. subglutinans* and *F. proliferatum* are also important (Munkvold, 2003). Some of the most important plant pathogenic fungal species known today are members of this genus. It is of concern that a large number of economically important plant species worldwide are susceptible to at least one or more *Fusarium* species (Lesli and Summerell, 2006).

Within the scope of this study, *Fusarium* species, which includes the rampant mycotoxin producing disease agents of maize, were explored in the Central and Southeastern Anatolia regions of Türkiye. The host specialization mechanisms of the agents have been supported by cross-pathogenicity studies.

MATERIALS AND METHODS

Field studies and *Fusarium* spp. isolations

During survey studies conducted in maize cultivation areas, plants exhibiting leaf yellowing and vascular browning symptoms at the 8–12 leaf stage (tassel and ear formation) were collected in paper bags (Fig. 1) from Eskişehir, Konya, and Karaman provinces (Central Anatolia) and Gaziantep, Şanlıurfa, Mardin, and Diyarbakır provinces (Southeastern Anatolia) (Fig. 2) during two growing seasons (August–October, 2021–2022), and subsequently brought to the laboratory for analysis. Crown rot sections (0.5–1 cm^2) from symptomatic plants were surface sterilized with 1.5% NaOCl (plus 0.5 ml/L Tween 20) for 3 minutes, then rinsed three times with sterile H_2O for 3 minutes each. After drying for 3–4 hours between sterile papers, tissues were placed on ½ PDA medium in standard Petri plates. Cultures were incubated at 25±1°C under white fluorescent light for one week. *Fusarium* spp. identification was performed by subculturing onto FMM (*Fusarium* Minimal Media), PSA (Potato Sucrose Agar), and SNA

(Synthetic Nutrient Agar) media (Can et al., 2003). For precise identification and pathogenicity testing, single-spore cultures were used to assess the genetic relatedness of isolates.

Identification of *Fusarium* spp. isolates

Species identification of *Fusarium* spp. was made according to Ismail et al., (2015), all samples of *Fusarium* spp. were subcultured in two different media, PSA and SNA, and incubated at 26±2 ºC for 8-10 days in order to properly reveal the morphological characters. *Fusarium* isolates were determined by taking into account the colony morphology of the samples developed at the end of incubation, mycelium development type, colony color, structure of microconidia and macroconidia, ability to form aerial micelles, clustering and chaining in microconidia, chlamydospore formation, sporodochium formation, and phialid structures. The species-level identification of *Fusarium* isolates was performed through microscopic examination using synoptic diagnostic keys (Booth, 1971; Nelson et al., 1983; Burgess et al., 1994; Seifert, 1996; Leslie and Summerell, 2006; Crous et al., 2021).

Pathogenicity studies

The seedling root dipping method was used in the pathogenicity test (Biles and Martyn, 1989). Roots of 2 weeks old susceptible maize variety P31G98 were immersed in the spore suspension at a concentration of $1x10^6$ conidia/ml and waited for 5 minutes (Altınok and Can, 2010). Planting of the inoculated seedlings was carried out in 3 replicates, with 3 plants in each pot. Disease severity % (DS) of seedlings was calculated according to the formula of Townsend and Heuberger (1943), the 0-4 scale (0: healty plants; 1: beginning of wilting, discoloration of fine veins on lower leaves; 2: wilting, growth retardation, chlorosis and necrosis in half of the plant; 3: general wilting, drying of leaves, shedding and death from the tips; 4: withering and death) (Altınok and Kamberoğlu, 2005).

For root scoring, plants were removed after four weeks and scored using a 0-4 scale modified from Muyolo et al. (1993) (0: no lesions and normal root length, 1: localized tissue discoloration and near normal root length, 2: extensive lesions and discoloration with root growth retardation, 3: severe root rot, limited root length, 4: complete root rot).

Pathogenic isolates were grouped under 5 categories [1: non pathogen (NP); 2: 1-20 % DS, weak pathogen (WP); 3: %21-50 DS, moderately pathogenic (MP); 4. 51-70% DS, strongly pathogenic (SP); 5. 70% < DS, highly pathogenic (HP)] according to the % DS values (Haware and Nene, 1982).

Molecular analyzes

DNA isolation of the isolates was performed from *Fusarium* cultures propagated from single spore isolation in Potato Dextrose Broth (PDB). DNA isolation was carried out manually by crushing the mycelial tissue taken from the propagated *Fusarium* cultures. RPB (RNA polymerase) and EF-1 alpha (Elongation Factor-1alpha) regions of genomic DNAs were amplified by PCR and the ~700 bp for EF-1 alpha and ~1100 bp for RPB fragments were subjected to electrophoresis (O'Donnell et al., 2015; Karlsson et al., 2016). By sequence analysis, the base sequences of the relevant regions of the DNA fragments were determined. Molecular identification was performed by comparing the obtained data with the sequences in the NCBI database with BLAST analysis.

Determination of host specificity mechanism

Cross-pathogenicity studies were conducted to assess the specificity of *Fusarium* spp. isolates to maize. Barley (cv. Crystal), wheat (cv. Ceyhan 99), common beans (cv. Canıpek), maize (cv. P31G98), garlic (cv. Chinese), sorghum (cv. Yeşilsoy), oat (cv. Kahraman) seeds that are in crop rotation with maize were used in the survey areas in this study. Virulent isolates of *F. proliferatum, F. subglutinans, F. verticillioides,* and *M. phaseolina* were selected for the studies. Agar plugs from 4–8 day-old PDA cultures were transferred to bottles containing sterilized oat seeds and incubated at 24±2 °C for 1–2 weeks, shaken daily for inoculum uniformity. In 1.5-liter pots, a 13 cm layer of a peat, perlite, and sand mixture (2:1:1) was placed, and 2 g of oat inoculum was mixed into each pot (Özgönen, 2011). While the fungus inoculum was mixed into the soil, each fungal species was mixed individually and in pairs and the pots then filled with a mixture of peat, perlite and sand (2:1:1). Sterilized seeds were sown at a depth of 1–2 cm and incubated at 26±2 °C and 70% humidity under 12:12 h dark/light conditions in a climate chamber. The experiment was repeated twice with three replicates, each containing three plants per pot. Disease symptoms and severity were monitored by comparing inoculated plants with controls. Scoring was performed every three days using the 0–4 scale described in the pathogenicity analysis section, and disease severity was calculated as a percentage. Statistical analysis was performed using Duncan's multiple comparison test ($p<0.05$) in the SPSS software.

RESULTS

Plants exhibiting disease symptoms were collected during surveys carried out on an area of approximately 99.000 decares in 290 corn growing fields in the Central Anatolia and

Southeastern regions, in the provinces of Eskişehir, Konya, Karaman, Mardin, Diyarbakır, Şanlıurfa, and Gaziantep (Fig. 2).

Pathogenicity studies of 115 isolates identified as *Fusarium* spp. of the samples collected from the survey area have been completed. The pathogenicity status was determined by comparing the plants inoculated with the root dipping method with the control plant (Fig. 1). Of the 31 isolates identified as pathogenic by pathogenicity studies, 28 were identified at the molecular level by sequencing studies. While the isolates had moderate pathogenicity in the stem of the cv P31G98, they exhibited weak pathogenicity in the roots (Table 1) and the % AUDPC values formed 7 groups (Fig. 3). Comparison of sequence data from 28 isolates using BLAST analysis in the NCBI database identified the common isolates as *F. proliferatum*, *F. subglutinans*, and *F. verticillioides*, all belonging to the *Fusarium fujikuroi* species complex (FFSC) group (Fig. 4).

When wilt symptoms were examined within the scope of cross pathogenicity studies, the fungal species were found to be weakly pathogenic on barley and wheats; moderately or strongly pathogenic on sorghum, maize and bean; moderate, strong and highly pathogenic in oat. All the fungal species were high pathogenic effect on garlic plants.

Garlic and oats were the most affected plant species. *M. phaseolina* exhibited strong pathogenicity on garlic, as did all other fungal groups. The highest disease severity in garlic was caused by the *F. proliferatum* + *F. verticillioides* combination, while in oats, it was caused by *M. phaseolina* + *F. verticillioides*. Although *M. phaseolina* alone caused the lowest disease severity in all plant groups, the combination of *M. phaseolina* + *F. verticillioides* (except in garlic) led to the highest severity across all plants.

Disease severity increased when *M. phaseolina* was combined with *Fusarium* species. Double inoculations often caused higher or comparable severity to single inoculations. Among *Fusarium* species, *F. verticillioides* caused the highest, and *F. subglutinans* (except in corn) the lowest disease severity on aboveground parts (Table 2, Fig. 5).

In parallel with wilt symptoms, the plant species most affected by fungal species were garlic and oats, while barley and wheat were the least affected plants. While inoculation of *M. phaseolina* alone caused the lowest disease severity in all plant groups, the combination of *M. phaesolina* + *F. verticillioides* (except garlic) caused the highest disease severity in all plants. It was observed that the disease severity in plants increased when *M. phaseolina* was inoculated with *Fusarium* species.

Upon examining the effects of single inoculations of *Fusarium* species on the roots of all plant species, *F. verticillioides* was found to cause the highest disease severity (except in oat and sorghum), while *F. subglutinans* resulted in the lowest severity (Table 3, Fig. 5).

When the general average of wilt and root rot disease severities shown by all inoculations in plants is examined, it was observed that the least affected plant was barley, and the most sensitive plant was garlic. Upon evaluating the overall average disease severity caused by fungal pathogens across plant groups, it was found that the lowest pathogenic effect resulted from *M. phaseolina* inoculation, while the highest effect occurred with *M. phaseolina* + *F. verticillioides* inoculation.

When the first symptom developments after inoculation (dai) in plants are examined, the earliest symptom development was in garlic from the 9th dai, and that started to be seen in barley and wheat plants as of the 21st dai at the latest. The first symptoms in the corn plant were from the 15th dai, starting from the 18th day in all inoculations on bean plants (except *M. phaseolina*); Starting from the 15th dai in all inoculations in sorghum (except *M. phaseolina*), started to develop on the oat plant from the 12th dai in all inoculations. While garlic was the plant most affected by the fungal species, barley was the least affected. The variation in disease severity among plants is attributed to factors such as genetic structure and defense mechanisms. Plant resistance to diseases and stresses is regulated by genetic factors through defense mechanisms like PR proteins (Van Loon et al., 2006), antioxidants (Mittler, 2002), phytoalexins (Hammerschmidt, 1999), phenolic compounds (Dixon and Paiva, 1995), and defense enzymes (Płażek, 2013). Bangar et al. (2022) reported a significant increase in total flavonoid and phenolic content, antioxidant activity, and metal chelating activity during the germination of various barley varieties. Flavonoids, known for inhibiting fungal root pathogens, act as antimicrobial agents (Makoi et al., 2007). High levels of antioxidant activity and flavonoid content were observed in germinated barley (Farooqui et al., 2018). Studies on barley mutants indicated that proanthocyanidins or dihydroquercetin contribute to *Fusarium* sp. resistance by inhibiting pathogen enzymes and/or reinforcing cell walls, forming a physical barrier (Skadhauge et al., 1997). The expression and secretion of certain PR proteins into intercellular spaces help control pathogen development through antimicrobial effects (Eslahi et al., 2021). In *Allium* plants, PR gene transcription is activated against *Fusarium* fungi. Significant differences in PR gene expression between *Fusarium*-resistant and susceptible garlic varieties (*A. sativum*) were observed (Anisimova et al., 2021). Palmero et al. (2013) found *F. proliferatum* pathogenic across 17 commercial cultivars, with higher susceptibility in white and

Chinese cultivars (81.84 ± 16.44% and 87.5 ± 23.19% symptomatic teeth, respectively) than in purple cultivars (49.06 ± 13.42%). Disease symptoms generally observed in plants were lodging, wilting, drying, root growth retardation, browning, root rot, and germination problems (Figs. 1, 6, 7).

DISCUSSION

In this study, among the *Fusarium* species identified as pathogens, the common species were defined *as F. proliferatum, F. subglutinans* and *F. verticillioides* (Table 1). According to literature records, *F. proliferatum, F. subglutinans* and *F. verticillioides* are among the *Fusarium* species that cause root- crown rot of maize plants (Munkvold and Leslie, 1999).

In maize, *F. verticillioides* and *F. proliferatum* produce fumonisin and trichothecene, *F. subglutinans* trigger fumonisin and moniliformin mycotoxins (Smith, 2001; Kabak and Var, 2005). *F. proliferatum*, *F. subglutinans, and F. verticillioides* species isolated from the research appear to have mycotoxin-producing capacity. When evaluated from this perspective, it is clear that additional studies are needed on these factors in line with the data obtained.

Fusarium species cause an average 26% loss in yield in cereal varieties commonly grown in Türkiye. It was stated that there was a 24% yield loss in bread wheat and 12% in barley (Hekimhan et al., 2005). Similar studies have also reported that *Fusarium* species cause diseases in cereals. For instance, in wheat-growing areas of Eskişehir, Türkiye, the prevalence of the disease has been found to reach up to 70% (Aktaş et al., 2000).

Fusarium spp. and *M. phaseolina* species cause diseases in beans in the world (Erper et al., 2008; Vural and Soylu 2012). In our study, these species isolated from maize were also found to be pathogenic in bean plants, exhibiting disease symptoms at rates varying between 11.11% and 59.72% (Tables 2, 3, Fig. 5). Similarly, to understand the host selection of *M. phaseolina*, Su et al., (2001) isolated *M. phaseolina* from the roots and soil of soybean, cotton, corn and sorghum plants, where the same plants were grown in the Los Angeles area for 15 years. While fungi isolated from maize plants caused higher disease symptoms in corn, no difference was detected in the severity of the disease caused in other plants. In addition, it was determined that isolates isolated from plants other than corn had no host selection. In our study, *M. phaseolina* caused disease severity at rates ranging from 2.78 to 62.50% on all plants in the experiment, without host selection (Tables 2, 3, Fig. 5).

Bağcı et al., (2010) reported that the sequential rotation application of beet + wheat + maize + wheat had the lowest disease severity. Lampkin (1997) reports that maize can form a good crop

rotation with wheat, barley and legumes. The disease severity of barley and wheat was lower than other plants used in this study. On the other hand, disease severity was found to be highest in garlic (Tables 2, 3). Therefore, rotating garlic in corn cultivation areas is considered a potential risk for production.

CONCLUSIONS

This study successfully identified *F. proliferatum*, *F. subglutinans*, and *F. verticillioides* as the predominant *Fusarium* species in the study area, with moderate pathogenicity in maize stems and weaker pathogenicity in roots, with disease severity reaching up to 50%. The cross-pathogenicity tests further revealed that these *Fusarium* species, along with *M. phaseolina* isolated from maize, exhibited varying levels of disease severity when inoculated in barley, wheat, bean, garlic, sorghum, and oat genotypes. These findings underscore the importance of selecting appropriate crop hosts for rotation to effectively manage soil-borne diseases.

ACKNOWLEDGEMENTS

We would like to thank the Gaziantep Scientific Research Projects Department (BAP) for their support of this study (Project Number: FEF.DT.22.10). The authors also thank the Republic of Türkiye, Ministry of Agriculture and Forestry, General Directorate of Agricultural Research and Policies (TAGEM) for support in field studies.

REFERENCES

Aktaş, A., Bolat, N., Keser, M. and İnce, T. 2000. Identification of wheat root rot pathogens in Eskişehir Province and determination of wheat and barley genotypes resistant to *Drechslera sorokiniana* (Sacc.) Subram. and Jain. *Plant Protection Bulletin*, **40(1-2)**: 71-83.

Altınok, H. H. and Can, C. 2010. Characterization of *Fusarium oxysporum* f. sp. *melongenae* isolates from eggplant in Turkey by pathogenicity, VCG, and RAPD analysis. *Phytoparasitica*, **38(2)**: 149-157.

Altınok, H. H. and Kamberoğlu, M. A. 2005. Prevalence and severity of *Fusarium* and *Verticillium* wilt diseases in eggplant production areas in Adana and Mersin provinces. *Çukurova University Journal of Agriculture Faculty*, **19(4)**: 1-8.

Anisimova, O. K., Shchennikova, A. V., Kochieva, E. Z. and Filyushin, M. A. 2021. Pathogenesis-Related Genes of PR1, PR2, PR4, and PR5 Families Are Involved in the

263 Response to *Fusarium* Infection in Garlic (*Allium sativum* L.). *International Journal of*
264 *Molecular Sciences*, **22**: 6688.
265 Aoki, T., O'Donnell, K. and Geiser, D. M. 2014. Systematics of key phytopathogenic *Fusarium*
266 species: current status and future challenges. *Journal of General Plant Pathology*, **80**:
267 189–201.
268 Bağcı, S. A., Hekimhan, H., Arısoy, R. Z., Taner, A., Büyük, O., Nicol, J. and Aydoğdu, M.
269 2010. Effect of different rotation systems on wheat root and crown rot disease. *Plant*
270 *Research Journal* **2**: 25–30.
271 Bangar, S. P., Sandhu, K. S., Trif, M., Manjunatha, V. and Lorenzo, J. M. 2022. Germinated
272 Barley Cultivars: Effect on Physicochemical and Bioactive Properties. *Food Analytical*
273 *Methods*, 1-8.
274 Biles, C. L. and Martyn, R. D. 1989. Local and systemic resistance induced in watermelons by
275 formae speciales of *Fusarium oxysporum*. *Phytopathology*, **79**: 856–860.
276 Booth, C., 1971. *The genus Fusarium*. Kew, England: Commonwealth Mycological Institute.
277 Burgess, L. W., Summerell, B. A., Bullock, S., Gott, K. P. and Backhouse, D. 1994. *Laboratory*
278 *manual for Fusarium research* (3rd ed.). Department of Crop Sciences, University of
279 Sydney.
280 Can, C., Elekçioğlu, H., Yücel, S. and Özaslan, M. 2003. Identification of *Fusarium* species
281 causing wilt in tomatoes in greenhouses, their relationship with nematodes in disease
282 development, and control options. TÜBİTAK (TOGTAG Project No: TARP-2371). Final
283 report.
284 Cengiz, R., 2016. Maize Research of Public Sector in Turkey, *Journal of Field Crops Central*
285 *Research Institute*, **25 (Special issue-1)**: 304-310.
286 Crous, P. W., Lombard, L., Sandoval-Denis, M. and et al. 2021. *Fusarium*: more than a node
287 or a foot-shaped basal cell. *Studies In Mycology*, **98**: 100-116.
288 Dixon, R. A., and Paiva, N. L. 1995. Stress-induced phenylpropanoid metabolism. *The Plant*
289 *Cell*, 7**(7)**: 1085-1097.
290 Early, R., 2009. *Pathogen control in primary production: crop foods. In: Foodborne*
291 *Pathogens*. 2nd Edition, Woodhead Publishing, UK. pp. 205-279.
292 Ekwomadu, T. I., 2019. *Occurrence and Variation of Fusarium Free and Masked Mycotoxins*
293 *in Maize from Agriculture Regions of South Africa*. Doctoral Dissertation, North-West
294 University, Potchefstroom, South Africa.

Erper, I., Karaca, G. H. and Özkoç, I. 2008. Root rot disease incidence and severity on some legume species grown in Samsun and the fungi isolated from roots and soils. *Archives of Phytopathology and Plant Protection*, **41(7)**: 501-506.

Eslahi, M. R., Safaie, N., Saidi, A., Shams-bakhsh, M., and Jafary, H. 2021. cDNA-AFLP analysis of plant defense genes expressed in wheat (cv. Chamran) infected with *Mycosphaerella graminicola*. *Journal of Agricultural Science and Technology*, **23(3)**: 699-710.

Farooqui, A. S., Syed, H. M., Talpade, N. N., Sontakke, M. D., and Ghatge, P. U. 2018. Influence of germination on chemical and nutritional properties of barley flour. *Journal of Pharmacognosy and Phytochemistry*, **7(2)**: 3855-3858.

Hammerschmidt, R., 1999. Phytoalexins: What have we learned after 60 years? *Annual Review of Phytopathology*, **37**: 285-306.

Haware, M. P. and Nene, Y. L. 1982. Races of *Fusarium oxysporum* f. sp. *ciceri*. *Plant Disease*, **66**: 809-810.

Hekimhan, H., Bağcı, S. A., Nicol, J. and Tunalı, B. 2005. Effects of root and root rot disease agents on some winter cereal yields. Proceedings of the VIth Field Crops Congress of Turkey, September 5–9, 2005, Research Presentation, Volume I, pp. 201–206, Antalya.

Ismail, M. A., Abdel-Hafez, S. I. I., Hussein, N. A. and Abdel-Hameed, N. A. 2015. Contributions to the genus *Fusarium* in Egypt with dichotomous keys for identification of species. *Szkółkarska*, **88B**, 62-002 Suchy Las, Poland.

Jain, P. K., Gupta, V. K., Misra, A. K., et al. 2011. Current status of *Fusarium* infection in human and animal. *Asian Journal of Animal and Veterinary Advances*, **6**: 201–227.

Kabak, B. and Var, I. 2005. *Fusarium* toxins. *Food and Feed Science and Technology*, **8**: 30-42.

Karlsson, I., Edel-Hermann, V., Gautheron, N., Durling, M. B., Kolseth, A. K., Steinberg, C., Persson, P. and Friberg, H. 2016. Genus-specific primers for study of *Fusarium* communities in field samples. *Applied and Environmental Microbiology*, **82**: 491–501.

Lampkin, N., 1997. *Organic farming* (Reprint ed.). Ipswich: Farming Press Books.

Leslie, J. F. and Summerell, B. A. 2006. *The Fusarium Laboratory Manual*. Blackwell Publishing: Hoboken, NJ, ABD.

Ma, L. J., Geiser, D. M., Proctor, R. H., Rooney, A. P., O'Donnell, K., Trail, F., Gardiner, D. M., Manners, J. M. and Kazan, K. 2013. *Fusarium* Pathogenomics. *Annual Review of Microbiology*, **67**: 399-416.

328 Makoi, J. H. J. R. and Ndakidemi, P. A. 2007. Biological, ecological and agronomic
329 significance of plant phenolic compounds in rhizosphere of the symbiotic legumes.
330 *African Journal of Biotechnology*, **6**: 1358–1368.
331 Mittler, R., 2002. Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science*,
332 7**(9)**: 405-410.
333 Munkvold, G., 2003. Epidemiology of *Fusarium* diseases and their mycotoxins in maize ears.
334 *European Journal of Plant Pathology*, **109(7)**: 705-713.
335 Munkvold, G. P. and Leslie, J. F. 1999. *Fusarium root rot diseases*. In D. G. White (Ed.),
336 *Compendium of corn diseases* (3rd ed., p. 13). The American Phytopathological Society.
337 Muyolo, N. G., Lipps, P. E. and Schmitthenner, A. F. 1993. Anastomosis grouping and variation
338 in virulence among isolates of *Rhizoctonia solani* associated with dry bean and soybean
339 in Ohio and Zaire. *Phytopathology*, **83(4)**: 438-444.
340 Nelson, P. E., Toussoun, T. A. and Marasas, W. F. O. 1983. *Fusarium species: An illustrated*
341 *manual for identification*. Pennsylvania State University Press.
342 O'Donnell, K., Ward, T. J., Robert, V., Crous, P. W., Geiser, D. M. and Kang, S. 2015. DNA
343 sequence-based identification of *Fusarium*: Current status and future directions.
344 *Phytoparasitica*, **43(5)**: 583–595.
345 Özgönen, H., 2011. Effects of arbuscular mycorrhizal fungi on plant growth and *Verticillium*
346 wilt (*Verticillium dahliae* Kleb.) in cotton. *Süleyman Demirel University, Journal of*
347 *Science Institute*, **15(3)**: 171-177.
348 Palmero, D., Gálvez, L., García, M., Gil-Serna, J. and Benito, S. 2013. The effects of storage
349 duration, temperature and cultivar on the severity of garlic clove rot caused by *Fusarium*
350 *proliferatum*. *Postharvest Biology and Technology*, **78**: 34–39.
351 Płażek, A. 2013. Induced resistance in plants to fungal diseases. *Postępy Nauk Rolniczych*, **6(1)**:
352 59-75.
353 Saremi, H. and Burgess, L. W. 2000. Effect of Soil Temperature on Distribution and Population
354 Dynamics of *Fusarium* Species. *Journal of Agricultural Science and Technology*, **2(2)**:
355 119-125.
356 Seifert, K. 1996. *Fuskey-Fusarium interactive key*. Agriculture and AgriFood Canada.
357 Skadhauge, B., Thomsen, K. K. and Von Wettstein, D. 1997. The Role of the Barley Testa
358 Layer and its Flavonoid Content in Resistance to *Fusarium* Infections. *Hereditas*, **126**:
359 147-160.
360 Smith, J. E. 2001. *Mycotoxins*. In *Food Chemical Safety* **1**:332.

Su, G., Suh, S. O., Schneider, R. W. and Russin, J. S. 2001. Host specialization in the charcoal rot fungus, *Macrophomina phaseolina*. *Phytopathology*, **91(1)**: 120-126.

Townsend, G. R. and Heuberger, I. W. 1943. Methods of estimating losses caused by diseases in fungicide experiments. *The Plant Disease Reporter*, **27**: 340–343.

Turkish Statistical Institute. (n.d.). Retrieved May 10, 2024, from https://www.tuik.gov.tr/

U.S. Department of Agriculture (USDA). (n.d.). Retrieved January 30, 2024, from https://www.usda.gov/

Van Loon, L. C., Rep, M. and Pieterse, C. M. J. 2006. Significance of induced resistance in plants for sustainable crop protection. *Annual Review of Phytopathology*, **44**: 309-332.

Vural, S. and Soylu, S. 2012. Prevalence and incidence of fungal disease agents affecting bean (*Phaseolus vulgaris* L.) plants. *Research on Crops*, **13(2)**: 634-640.

Yıldırım, A. F., Araz, A., Turgay, B., Ünal, F. and Büyük, O. 2016. *Root and Root Collar Past, Present and Struggle in Cool Climate Cereals*, General Directorate of Agricultural Research and Policies, Agricultural Control Central Research Institute. 1-104.

375 **Table 1.** List of virulent isolates.

#	Province	District	Neighbourhood	Isolate code	Wilt Disease severity (%)	Root Disease Severity (%)	Species	NCBI Banklt No
1	Diyarbakır	Centre City	Karamusa	F21MK1/1-21	44,44 (MP)	13,89 (WP)	*F. proliferatum*	PP782625
2				F21MK1/2-21	47,22 (MP)	13,89 (WP)		PP782626
3	Eskişehir	Tepebaşı	Gündoğdu	F26TG1/1-21	38,89 (MP)	16,67 (WP)	*F. verticillioides*	PP782616
4				F26TG1/2-21	47,23 (MP)	11,11 (WP)	*F. proliferatum*	PP782627
5	Gaziantep	Nurdağı	Kömürler	F27NK2/2-21	47,23 (MP)	16,67 (WP)	*F. verticillioides*	PP782617
6		Araban	Dağdancık	F27AD1/3-21	41,67 (MP)	16,67 (WP)	*F. verticillioides*	PP782618
7				F27AD2/1-21	38,89 (MP)	13,89 (WP)		PP782619
8			Güllüce	F27AG2/3-21	44,44 (MP)	13,89 (WP)	*F. verticillioides*	PP782620
9	Konya	Çumra	Küçükköy	F42ÇK1/2-21	50,00 (MP)	16,67 (WP)	*F. verticillioides*	PP782621
10			Abditolu	F42ÇA1/2-21	38,89 (MP)	13,89 (WP)	*F. verticillioides*	PP782622
11				F42ÇA1/3-21	50,00 (MP)	16,67 (WP)	*F. proliferatum*	PP782628
12		Karatay	Erler	F42KE1/3-21	50,00 (MP)	16,67 (WP)	*F. proliferatum*	PP782629
13	Mardin	Derik	Soğukkuyu	F47DS1/2-21	41,67 (MP)	13,89 (WP)	*F. proliferatum*	PP782630
14		Artuklu	Yolbaşı	F47AY1-21	38,89 (MP)	8,33 (WP)	*F. subglutinans*	PP817195
15		Derik	Ahmetli	F47DA1/1-21	44,44 (MP)	8,33 (WP)	*F. subglutinans*	PP817196
16	Şanlıurfa	Halilliye	Boydere	F63HB1/3-21	44,44 (MP)	8,33 (WP)	*F. subglutinans*	PP817197
17				F63HB1/2-21	44,44 (MP)	8,33 (WP)		PP817199
18		Akçakale	Şanlı	F63AŞ1/1-21	41,67 (MP)	5,56 (WP)	*F. subglutinans*	PP817198
19	Karaman	Centre City	Yollarbaşı	F70MY1/7-21	44,44 (MP)	8,33 (WP)	*F. subglutinans*	PP817200
20				F70MY2/1-21	44,44 (MP)	8,33 (WP)	*F. subglutinans*	PP817201
21				F70MY2/2-21	44,44 (MP)	5,56 (WP)	*F. proliferatum*	PP782631
22			Sudurağı	F70MS1-21	41,67 (MP)	11,11 (WP)	*F. proliferatum*	PP782632
23			Piri Reis	F70MP2/1-21	44,44 (MP)	16,67 (WP)	*F. verticillioides*	PP782623
24			Hamidiye	F70MH2/4-21	38,89 (MP)	13,89 (WP)	*F. proliferatum*	PP782633
25				F70MH2/6-21	44,44 (MP)	16,67 (WP)		PP782634
26				F70MH2/7-21	47,22 (MP)	11,11 (WP)	*F. subglutinans*	PP817202
27	Diyarbakır	Çınar	Yuvacık	F21ÇY3-22	41,67 (MP)	16,67 (WP)	*F. verticillioides*	PP782624
28	Eskişehir	Odunpazarı	Ağapınar	F26OA2-22	44,44 (MP)	13,89 (WP)	*F. proliferatum*	PP782635

376 MP: Moderately pathogenic, WP: Weakly pathogenic.

Table 2. Wilt disease severity (%) values and pathogenicity groups (Duncan p<0.05).

Species	Barley*	Wheat	Oat	Sorghum	Maize	Bean	Garlic
M. phaseolina	5,56 ab (WP)	6,94 a (WP)	37,50 a (MP)	34,72 a (MP)	37,50 a (MP)	34,72 a (MP)	62,50 a (SP)
F. proliferatum	2,78 a (WP)	9,72 abc (WP)	52,78 ab (SP)	50,00 b (MP)	43,06 ab (MP)	34,72 a (MP)	73,61 ab (HP)
F. subglutinans	2,78 a (WP)	6,94 a (WP)	50,00 ab (MP)	45,83 ab (MP)	45,83 abc (MP)	34,72 a (MP)	70,83 ab (HP)
F. verticillioides	12,50 bc (WP)	13,89 abc (WP)	56,94 ab (SP)	51,39 b (SP)	51,39 bc (SP)	40,28 a (MP)	75,00 ab (HP)
M. phasolina + *F. proliferatum*	12,50 bc (WP)	15,28 bc (WP)	73,61 b (HP)	56,94 b (SP)	55,56 bc (SP)	56,94 b (SP)	77,78 ab (HP)
M. phaseolina + *F. subglutinans*	8,33 abc (WP)	8,33 ab (WP)	62,50 ab (SP)	54,17 b (SP)	56,94 c (SP)	59,72 b (SP)	73,61 ab (HP)
M. phaesolina + *F. verticillioides*	15,28 c (WP)	16,67 c (WP)	76,39 b (HP)	56,94 b (SP)	58,33 c (SP)	59,72 b (SP)	77,78 ab (HP)
F. proliferatum + *F. subglutinans*	9,72 abc (WP)	11,11 abc (WP)	55,56 ab (SP)	51,39 b (SP)	48,61 abc (MP)	45,83 ab (MP)	70,83 ab (HP)
F. proliferatum + *F. verticillioides*	11,11 abc (WP)	12,50 abc (WP)	59,72 ab (SP)	51,39 b (SP)	55,56 bc (SP)	48,61 ab (MP)	84,72 b (HP)
F. subglutinans + *F. verticillioides*	5,56 ab (WP)	11,11 abc (WP)	70,83 b (HP)	48,61 b (MP)	54,17 bc (SP)	48,61 ab (MP)	76,39 ab (HP)

NP: Non-pathogenic WP: Weakly pathogenic MP: Moderately pathogenic SP: Strongly pathogenic HP: Highly pathogenic

* The same letters in each column indicate different groups in the Duncan test (p<0.05)

Table 3. Root disease severity (%) values and pathogen groups.

Species	Barley*	Wheat	Oat	Sorghum	Maize	Bean	Garlic
M. phaseolina	2,78 a (WP)	2,78 a (WP)	37,50 a (MP)	23,61 a (MP)	12,50 a (WP)	13,89 a (WP)	56,94 a (SP)
F. proliferatum	2,78 a (WP)	5,56 ab (WP)	50,00 ab (MP)	37,50 ab (MP)	15,28 a (WP)	16,67 a (WP)	63,89 ab (SP)
F. subglutinans	2,78 a (WP)	2,78 a (WP)	41,67 ab (MP)	33,33 ab (MP)	11,11 a (WP)	11,11 a (WP)	58,33 ab (SP)
F. verticillioides	11,11 ab (WP)	11,11 ab (WP)	48,61 ab (MP)	34,72 ab (MP)	15,28 a (WP)	16,67 a (WP)	68,06 ab (SP)
M. phasolina + *F. proliferatum*	8,33 ab (WP)	8,33 ab (WP)	61,11 ab (SP)	43,06 b (MP)	22,22 a (MP)	22,22 a (MP)	62,50 ab (SP)
M. phaseolina + *F. subglutinans*	6,94 ab (WP)	2,78 a (WP)	52,78 ab (SP)	40,28 b (MP)	19,44 a (WP)	20,83 a (MP)	61,11 ab (SP)
M. phaesolina + *F. verticillioides*	13,89 b (WP)	13,89 b (WP)	70,83 b (HP)	43,06 b (MP)	23,61 a (MP)	22,22 a (MP)	72,22 ab (HP)
F. proliferatum + *F. subglutinans*	2,78 a (WP)	4,17 ab (WP)	48,61 ab (MP)	33,33 ab (MP)	15,28 a (WP)	15,28 a (WP)	65,28 ab (SP)
F. proliferatum + *F. verticillioides*	6,94 ab (WP)	8,33 ab (WP)	45,83 ab (MP)	37,50 ab (MP)	19,44 a (WP)	19,44 a (WP)	73,61 b (HP)
F. subglutinans + *F. verticillioides*	4,17 ab (WP)	5,56 ab (WP)	59,72 ab (SP)	33,33 ab (MP)	18,06 a (WP)	18,06 a (WP)	70,83 ab (HP)

NP: Non-pathogenic WP: Weakly pathogenic MP: Moderately pathogenic SP: Strongly pathogenic HP: Highly pathogenic

* The same letters in each column indicate different groups in the Duncan test (p<0.05)

Figure 1. Symptom exhibiting maize plants in the field. a) General view of *Fusarium* spp. infected maize field; b) Maize plants exhibiting symptoms; c) Stem sections of diseased maize plants; Disease symptoms: d) Control plant (left) and the plant showing wilting-yellowing symptoms (right); e-f) Root symptoms; Root and crown symptoms developing in plants: g-h) garlic, i-j) maize, k-l) wheat, m-n) sorghum, o) beans, p) barley.

Figure 2. Provinces in Türkiye where survey was conducted.

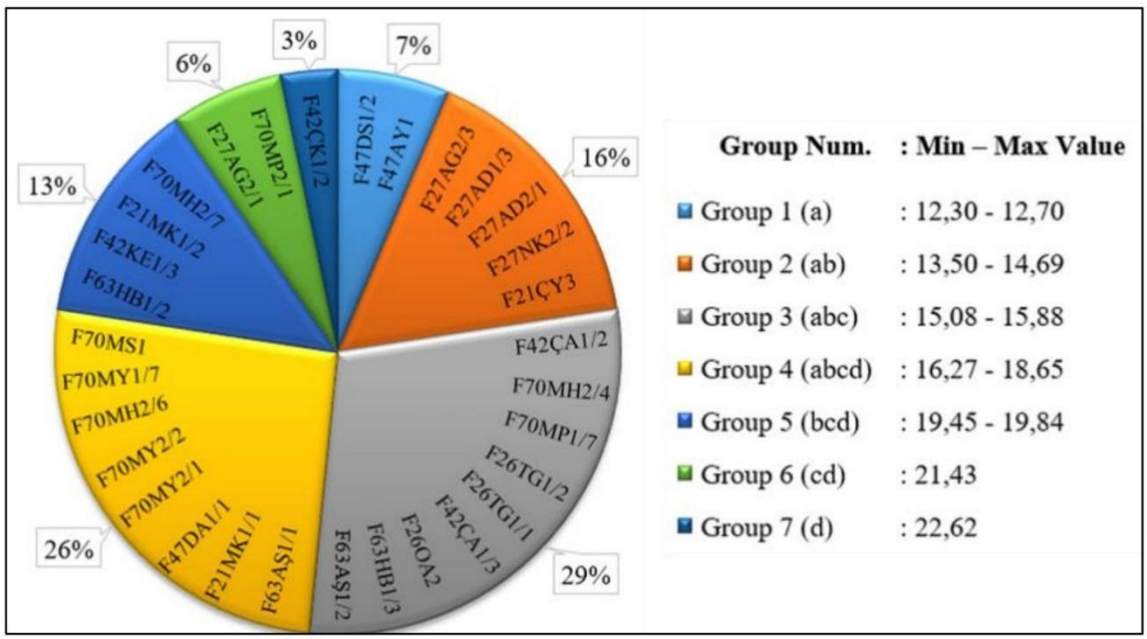

Figure 3. Wilting AUDPC % values (Duncan P< 0.05).

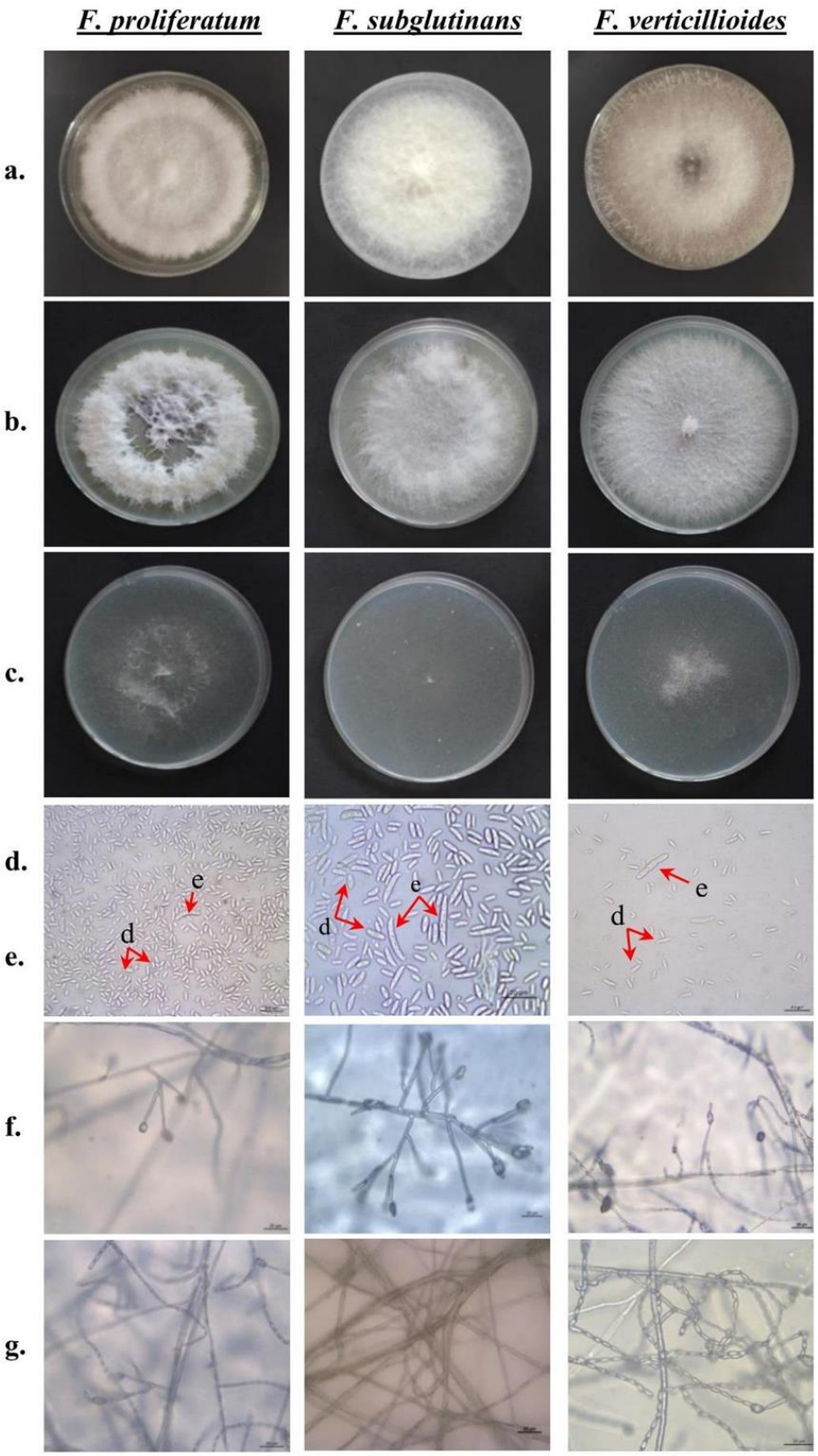

Figure 4. Cultural and morphological characteristics of *Fusarium* species a) Growth in PDA medium, b) Growth in PSA medium, c) Growth in SNA medium, d) Microconidia, e) Macroconidia, f) Phialid structures, g) Chain structures (It is present in *F. proliferatum* and *F. verticillioides*, is absent in *F. subglutinans*) (Scale: 20µm).

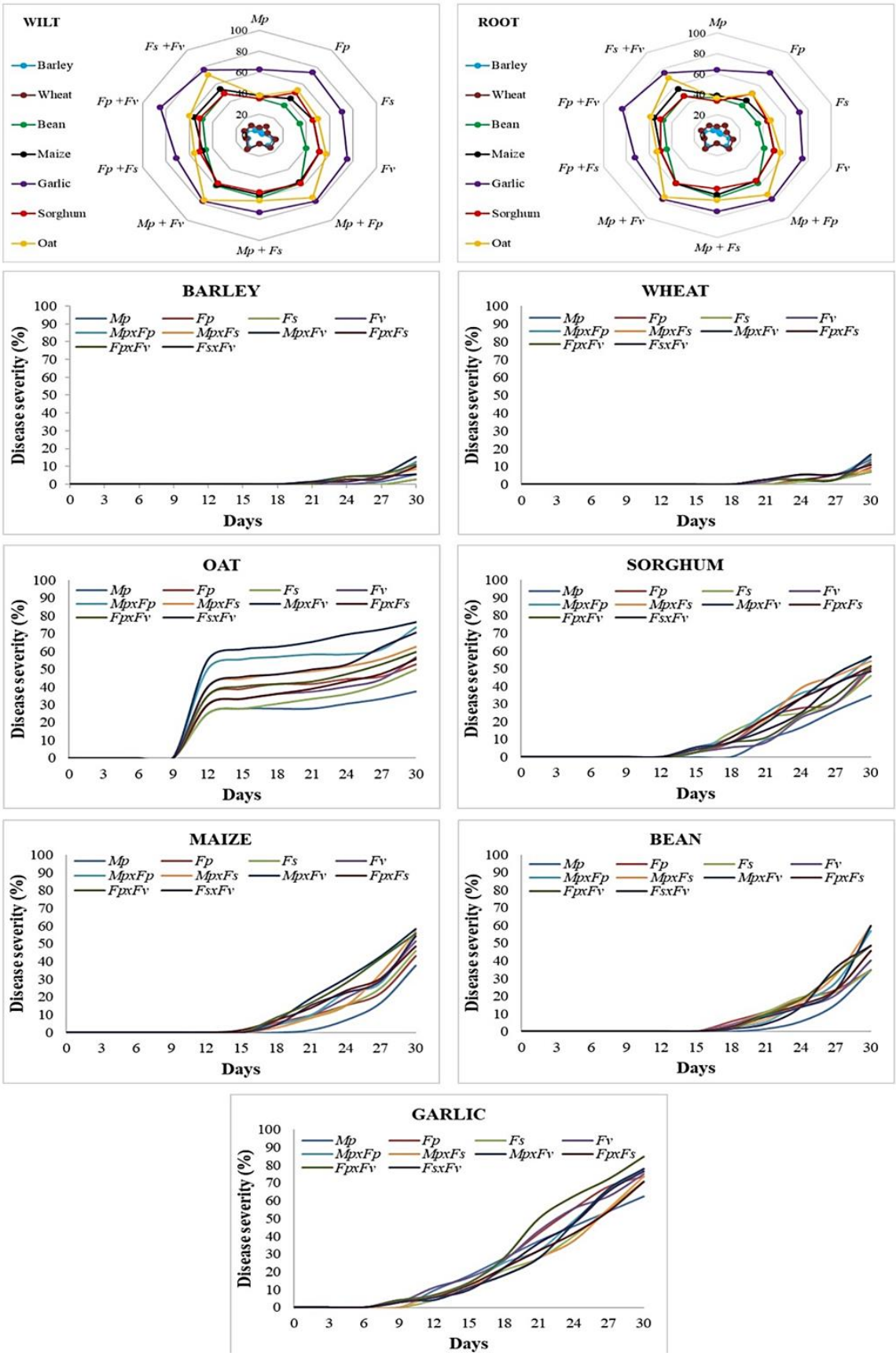

Figure 5. Disease severity (%) values and symptom development in plants (% disease severity/day).

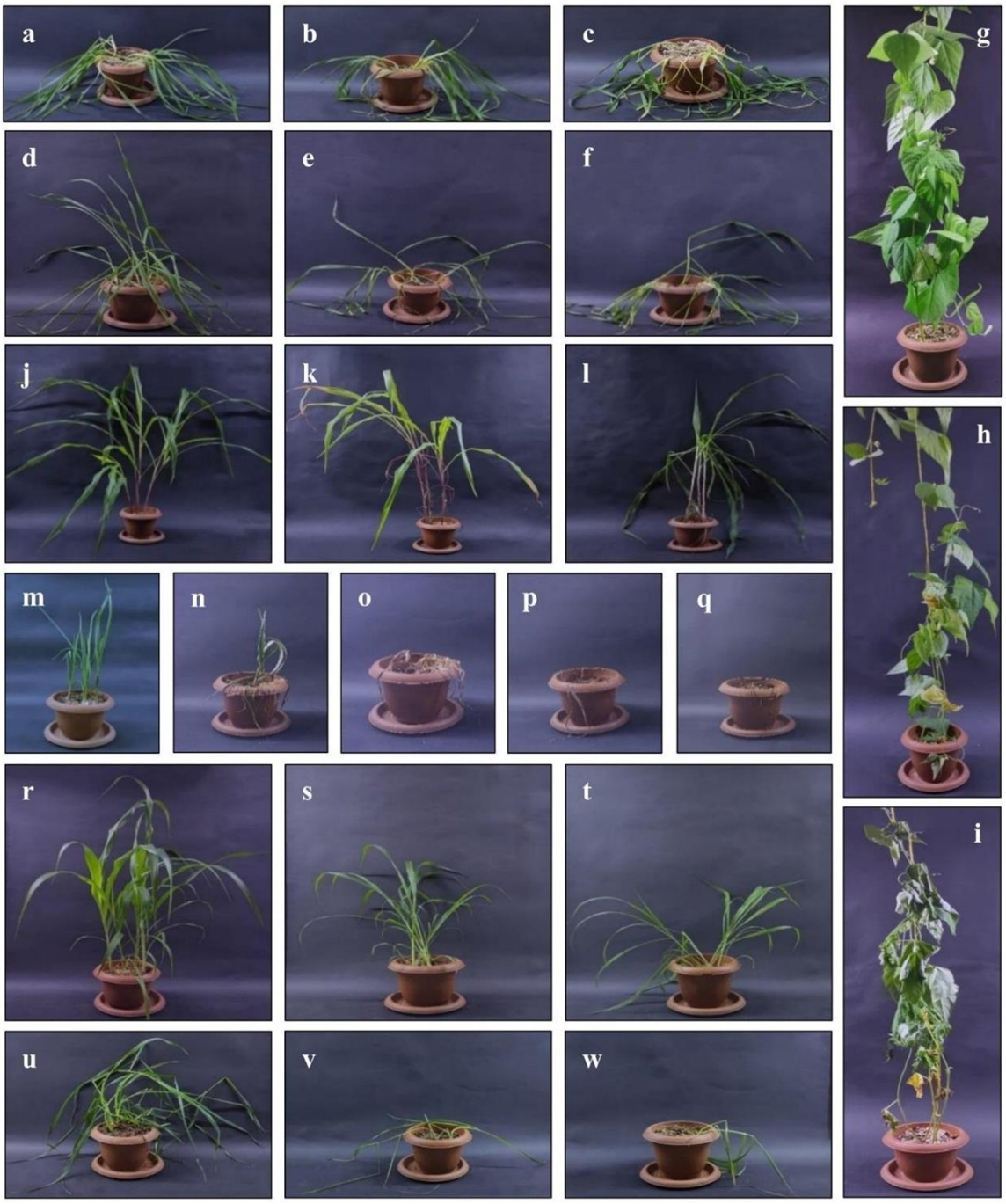

Figure 6. Disease symptoms in plants: Barley: a. Control, b. *Fp*, c. *Fp+Fv*; Wheat: d. Control, e. *Mp*, f. *Mp+Fp*; Beans: g. Control, h. *Mp*, i. *Fp+Fs*; Maize: j. Control, k. *Mp*, l. *Mp+Fv*; Garlic: m. Control, n. *Mp*, o. *Fs*, p. *Mp+Fs*, q. *Fp+Fv*; Sorghum: r. Control, s. *Mp*, t. *Mp+Fv*; Oats: u. Control, v. *Mp*, w. *Mp+Fv*. (*Mp: M.phaseolina, Fp: F.proliferatum, Fs: F. subglutinans, Fv: F. verticillioides*).

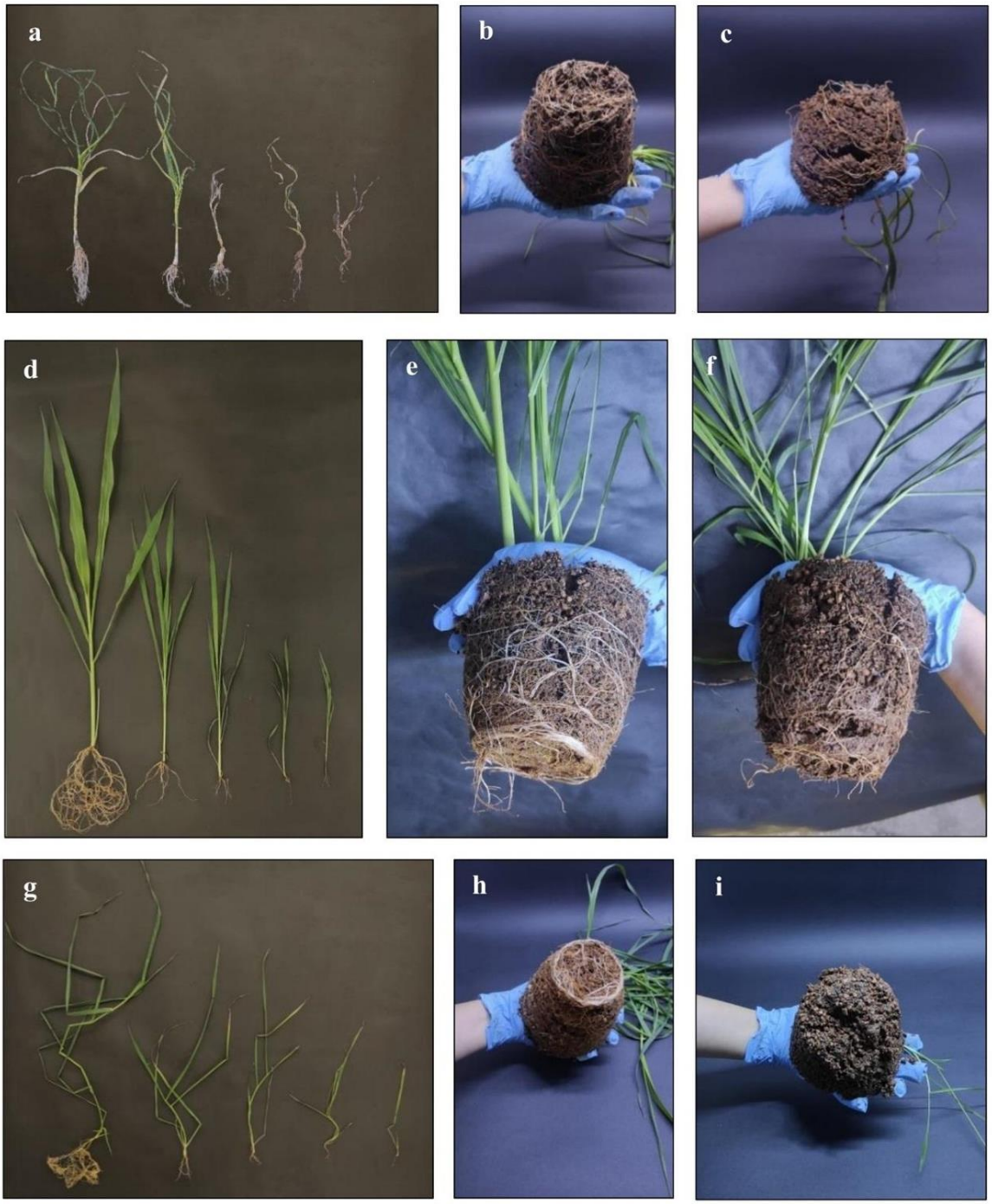

Figure 7. Scoring and root development of plants: Garlic: a. Scoring, b. Control plant root, c. *Fp+Fv* inoculated plant root; Sorghum: d. Scoring, e. Control plant root, f. *Mp+Fv* inoculated plant root; Oats: g. Scoring, h. Control plant root, i. *Mp+Fv* inoculated plant root. (*Mp: M.phaseolina, Fp: F.proliferatum, Fs: F. subglutinans, Fv: F. verticillioides*).