Assiut Journal of Agricultural Sciences 54 (2) 2023 (159-170) Website: http://ajas.journals.ekb.eg/
ISSN: 1110-0486 / EISSN: 2356-9840 E-mail: ajas@aun.edu.eg

(Original Article)

Molecular Identification of the Causal Pathogens of Faba Bean Leaf Spot Disease in Upper Egypt

Hend S.A. Shehata[*1]; Keinawi M.H. Abd-El-Moneem[1]; Mohsen E.A. Abo-Rehahb [2] and Amal M.I. Eraky [1]

[1]Department of Plant Pathology, Faculty of Agriculture, Assiut University, Assiut, Egypt.
[2] Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt.

*Corresponding author email: hendhend20001720@yahoo.com
DOI: 10.21608/ajas.2023.188224.1223

Abstract

Faba bean (*Vicia fabae*) is considered the most important legume crop in Egypt. Eighty fungal isolates were isolated from faba bean leaves cultivated in different localities in Assiut and Sohag governorates. All isolates were identified using morphological characteristics of mycelia and spores' features. These isolates were identified by molecular methods using ITS-rDNA sequencing (ITS1 and ITS4). A resulted based on ITS sequences the fungal samples were *Alternaria angustiovoidea* (OM432027), *Curvularia lunata* (OM432028) and *Fusarium solani* (OM432026). Pathogenicity tests were performed in a greenhouse on faba bean cultivar 843. Different degrees of leaf spot disease symptoms were produced by each tested isolate. *A. angustiovoidea C. lunata* and *F. solani* produced the highest disease severity percentage 62.33, 62.66 and 50.23%, respectively. Evaluation of four cultivars reaction to leaf spot disease showed that, Giza 843 was the most susceptible cultivar followed by Giza 125 while, the most tolerant cultivar was Giza 2 followed by Giza 667.

Keywords: *Vicia faba L., leaf spot, Alternaria angustiovodea, Curvularia lunata, Fusarium solani.*

Introduction

Faba bean (*Vicia fabae*) is considered the most important legume crop in Egypt. The cultivated area in 2020 was approximately 117309 feddan, yielding 168437 tones. Due to its great nutritional value in terms of calorie and protein contents (24–30%) and ability to be a good nitrogen fixer, it is one of the most significant dietary legumes (Sahile *et al.,* 2008). Faba bean seeds have a high concentration of important mineral components and may be a source of dietary fiber (Mahmoud, 1996; Reis *et al.,* 2007; Juroszek and von Tiedemann, 2011). Alternaria leaf spot is caused by *Alternaria alternata*. This disease of faba beans occurring late in the growing season as the plants start to mature. The main symptoms of this disease occurring's dark brown spots on the leaves which have a zoned brown ring with dark margin, the fungus probably survives on other hosts and crop residues (Hawatin and Webb, 1981; Simmons 2007).

Received: 30 January 2023/ Accepted: 29 March 2023/ Published online: 20 April 2023

Molecular characterization of rDNA regions using internal transcribe spacers (ITS) based on gene sequence analysis is useful in identifying and characterizing isolates of fungi as potential new species level and within species (White *et al.*, 1990).

The purpose of this study was to isolate and molecularly identify the pathogens that caused the leaf spot disease on faba beans in Upper Egypt as well as to assess how different faba bean cultivars responded to the infection.

Materials and Methods

Isolation of the causal pathogens

Samples of faba bean plants exhibiting the usual leaf spot signs of infected plants were gathered from several locations in Assiut and Sohag governorates. The surfaces of diseased leaves were sanitized by submerging them in 2% sodium hypochlorite solution for 2 minutes, cutting into small pieces, rinsing three times in sterilized distilled water for 2 minutes, and then drying with sterilized filter papers. On potato dextrose agar medium (PDA), the surface-sterilized plant pieces were put before being incubated at 25°C. After 4-5-day incubation period, the isolated fungi were purified on PDA medium using the hyphal tip and single spore techniques. The pure fungal isolates were then grown on PDA slants at 27°C and kept in the fridge at 4 °C for until used. (Dhingra and Sinclair, 1985).

Pathogenicity test

The experiment was carried out in Plant Pathology greenhouse, Faculty of Agriculture, Assiut University. Pots 25 cm in diameter were sterilized with 5% formaldehyde solution, filled with sterilized sand clay soil (3.5kg-pot). Four seeds were sown in each pot, and four pots were used as replicates for each isolate. Eighty fungal isolates were prepared by growing in 250 ml conical flasks containing 100 ml of liquid medium of potato dextrose for 15 days at 26°C.

Altogether, 80 isolates isolated from diseased faba bean leaves were employed in the pathogenicity test. The resulted suspension of each isolate was adjusted to concentration approximately 5×10^5 CFU/ ml (Rahman *et al.*, 2002). Plants were sprayed with mycelia and spore suspensions after 45 days of planting using sterilized atomizer. Spraying sterilized water on plants acted as a control. After inoculation, plants were sealed in plastic bags for 48 hours to maintain high moisture levels. Disease severity percentage was noticed after 15 days and 30 days from in the artificial infection. Diseases severity of leaf spot of faba bean was recorded and calculated using the arbitrary scale of 0 to 9. Where 0, 1, 2, 3, 4, 5, 6, 7 and 8 represent no visible leaf infection (0) or diseases covering less than 10%, 20%, 30%, 40%, 50%, 60%, 70% and 80 % of the foliar tissue, respectively; (9) represents disease covering more than 80 of the foliar tissue (EL-Hendawy *et al.*, 2010). Disease severity percentage calculated using the following formula:

$$\mathrm{DS}\ (\%) = \Sigma \mathrm{d} \,/\, (\mathrm{d\ max} \times \mathrm{n}) \times 100$$

Whereas DS: is the disease rating of each plant, d max is the maximum disease rating and n is the total number of plants examined in each replicate.

Identification of the eighty fungal isolates were carried out in Plant Pathology Department Facluty of Agriculture, Assiut University according to its morphological characters of mycelia and spores as described by Booth (1971) for *Fusarium,* Ellis (1976) for *Curvularia* and Simmons (2007) for *Alternaria.*

Molecular characterization of pathogenic isolates

The three fungal isolates causing the highest disease severity of leaf spot disease were identified using molecular methods (ITS-rDNA). The fungi were cultivated on Czapek`s yeast extract agar (CYA) medium (Pitt and Hocking (2009) and incubated at 28° C for 7 days. The fungal culture was sent to the Molecular Biology Research Unit, Assiut University for DNA extraction using pathogene-spin DNA/RNA extraction kit provided by Intron Biotechnology Company, Korea. The fungal DNA was kept in sterile eppendorf and then shipped to Sol Gent Company, Daejeon South Korea for polymerase chain reaction (PCR) sequencing. PCR was performed using two universal fungal primers ITS1 ((5' - TCC GTA GGT GAA CCT GCG G - 3') and ITS4 (5'- TCC TCC GCT TAT TGA TAT GC -3'). were sequenced in the sense and antisense directions using ITS1 and ITS4 primers (White *et al.*, 1990). The purified PCR products (amplicon) were reconfirmed using a size nucleotide marker (100 base pairs) by electrophoresis on 1% agarose gel. Then these bands were eluted and sequenced with the incorporation of did eoxynucleotides (dd NTPs) in the reaction mixture. The PCR products of the fungal samples were sequenced in the sense and antisense directions using ITS1 and ITS4 primers. DNA sequences of the three species in this study were assembled using the DNA STAR computer package (version 5.05). The three isolates' assembled sequences were aligned with those from GenBank using MAFFT. (Katoh and Standley, 2013). Alignment gaps and parsimony uninformative characters were treated by BMGE (Criscuolo and Gribaldo, 2010). Maximum – likelihood (ML) and Maximum parsimony (MP) phylogenetic analyses were performed using phyML 3.0 (Guindon *et al.*, 2010). The robustness of the most parsimonious trees was evaluated by 1000 bootstrap replications (Felsenstein, 1985). The best optimal model of nucleotide substitution for the ML analyses was determined using Smart Model Selection (SMS) version 1.8.1 (Lefort *et al*., 2017). The phylogenetic tree was visualized.

Reaction of certain faba bean cultivars to leaf spot disease under greenhouse conditions

Four faba bean cultivars (Giza 2, Giza 125, Giza 667 and Giza 843) obtained from (Department of Agronomy, Faculty of Agriculture, Assiut University, Egypt) were evaluated for their susceptibility to leaf spot disease caused by *(Alternaria angustiovodea, Curvularia lunata* and *Fusarium solani*) individually under greenhouse conditions in winter 2020 season. The highly pathogenic isolates (*Alternaria angustiovodea* No. 40*, Curvularia lunata* No. 59 and *Fusarium solani* No. 80) were selected for this experiment. Four seeds for each pot were cultivated.

Four replicates for each cultivar. Faba bean plants of each tested cultivar were sprayed after 45 days of cultivation with a spore concentration of each tested pathogenic isolate as previous mentioned in pathogenicity test. Spray with water only was used as a control (Rahman *et al*., 2002). Disease severity percentage was noticed after 15 days and then recorded after 30 days from spraying as mentioned before.

Statistical analysis

All Data were analyzed in a complete randomized design. Data subjected to analysis of variance carried out using (SAS computer version 9.0) according to SAS Institute Inc. Means were compered using Duncan's multiple range test at level $P \leq 0.05$ (Gomez and Gomez, 1984).

Results

Isolation and Identification the causal pathogens

Eighty fungal isolates were purified and morphologically identified according to their morphological characteristics (Table 1).

Pathogenicity test

Pathogenic capability of the isolated fungal isolates was carried out in open greenhouse in Pant Pathology Dept., Faculty of Agriculture, Assiut University. Data in Table 1 showed that, isolates No. 40, 59, and 80 caused the highest disease severity %, its exhibited 62.33, 62.66 and 50.33%, respectively. Among all 80 tested isolates, Alternaria sp. was the most common pathogenic isolates and almost 70% of isolates were *Alternaria* sp. (57/80) followed by *Curvularia* sp. (17/80) then *Fusarium* sp. (6/80). The rest of screened isolates had disease severity% ranging from 13.15 to 62.33%.

Molecular identification of pathogenic isolates

Molecular identification of the highly isolates was carried out using ITS. Isolates No. 40, 59 and 80, which shown the highest pathogenicity on faba bean plants, were selected for molecular identification using ITS-rDNA sequencing. The sequencing results revealed that these isolates were *Alternaria angustiovoidea*, *Curvularia lunata* and *Fusarium solani* respectively. These isolates were aligned individual with that most related isolates from GenBank to produce phylogenetic trees as show in Figs (1, 2 and 3). Isolate No. 40 (*Alternaria angustiovoidea* AUMC 15457 with GenBank accession No. OM432027, arrowed) aligned with closely related strains accessed from the GenBank. It showed 99.64% - 99.82% identity and 99% - 100% coverage with several strains of *A. angustiovoidea* including the type of strain, CBS 195.86 with GenBank accession No. MH861939.

Table 1. Pathogenicity test of 80 fungal isolates on faba beancultivar (cv843) under greenhouse conditions

Isolate No.	Pathogen	Disease severity (%)	Isolate No.	Pathogen	Disease severity (%)
1	*Alternaria* sp.	41.00^{uv*}	41	*Alternaria* sp.	55.33^{ec}
2	*Alternaria* sp.	36.12^{cd}	42	*Alternaria* sp.	57.33^{ec}
3	*Alternaria* sp.	29.00^{jh}	43	*Alternaria* sp.	52.31^{ef}
4	*Alternaria* sp.	40.45^{uv}	44	*Alternaria* sp.	57.33^{ec}
5	*Alternaria* sp.	38.13^{cd}	45	*Alternaria* sp.	59.61^{a}
6	*Alternaria* sp.	33.66^{jh}	46	*Alternaria* sp.	46.14^{op}
7	*Alternaria* sp.	19.33^{lm}	47	*Alternaria* sp.	48.21^{ef}
8	*Alternaria* sp.	38.12^{cd}	48	*Alternaria* sp.	50.23^{ef}
9	*Alternaria* sp.	52.23^{ef}	49	*Alternaria* sp.	36.22^{cd}
10	*Alternaria* sp.	25.00^{jk}	50	*Alternaria* sp.	50.12^{ef}
11	*Alternaria* sp.	20.33^{lm}	51	*Alternaria* sp.	48.12^{ef}
12	*Alternaria* sp.	22.45^{lk}	52	*Alternaria* sp.	41.11^{uv}
13	*Alternaria* sp.	53.00^{ef}	53	*Alternaria* sp.	33.14^{jh}
14	*Alternaria* sp.	13.15^{lm}	54	*Alternaria* sp.	43.12^{uv}
15	*Alternaria* sp.	31.45^{jh}	55	*Alternaria* sp.	50.33^{ef}
16	*Alternaria* sp.	34.33^{jh}	56	*Alternaria* sp.	35.00^{cd}
17	*Alternaria* sp.	35.33^{jk}	57	*Alternaria* sp.	33.02^{jh}
18	*Alternaria* sp.	43.11^{uv}	58	*Curvularia* sp.	25.02^{jk}
19	*Alternaria* sp.	33.44^{jh}	59	*Curvularia* sp.	62.66^{a}
20	*Alternaria* sp.	53.66^{ef}	60	*Curvularia* sp,	27.00^{jh}
21	*Alternaria* sp.	55.16^{ec}	61	*Curvularia* sp.	35.12^{cd}
22	*Alternaria* sp.	49.13^{ef}	62	*Curvularia* sp.	30.30^{jh}
23	*Alternaria* sp.	42.6^{uv}	63	*Curvularia* sp.	33.00^{jh}
24	*Alternaria* sp.	40.75^{uv}	64	*Curvularia* sp.	46.22^{op}
25	*Alternaria* sp.	34.33^{jh}	65	*Curvularia* sp.	47.12^{ef}
26	*Alternaria* sp.	35.33^{jh}	66	*Curvularia* sp.	40.13^{uv}
27	*Alternaria* sp.	42.22^{uv}	67	*Curvularia* sp.	44.15^{op}
28	*Alternaria* sp.	36.00^{cd}	68	*Curvularia* sp.	43.00^{uv}
29	Alternaria sp.	34.33^{jh}	69	*Curvularia* sp.	48.15^{ef}
30	Alternaria sp.	44.00^{op}	70	*Curvularia* sp.	36.61^{cd}
31	Alternaria sp.	45.11^{op}	71	*Curvularia* sp.	50.45^{ef}
32	Alternaria sp.	42.14^{uv}	72	*Curvularia* sp.	43.61^{uv}
33	*Alternaria* sp.	36.12^{cd}	73	*Curvularia* sp.	46.22^{op}
34	*Alternaria* sp.	46.34^{op}	74	*Curvularia* sp.	42.00^{uv}
35	*Alternaria* sp.	45.34^{op}	75	*Fusarium* sp.	45.45^{op}
36	*Alternaria* sp.	57.33^{ec}	76	*Fusarium* sp.	40.60^{uv}
37	*Alternaria* sp.	43.31^{uv}	77	*Fusarium* sp.	37.10^{cd}
38	*Alternaria* sp.	48.33^{ef}	78	*Fusarium* sp.	38.00^{cd}
39	*Alternaria* sp.	42.33^{uv}	79	*Fusarium* sp.	36.00^{cd}
40	*Alternaria* sp.	62.33^{a}	80	*Fusarium* sp.	50.23^{ef}
	Control	Water		0.00	

*Means in each column followed by the same letter are not significantly different according to Duncan's multiple range tests at 5%.

The fungal strain showed also close relationship with *Alternaria alternate*, *A. detrains* and *A. tenuissima* (Fig 1). Isolates No. 59 was (*Curvularia lunata* AUMC 15458 with GenBank accession No. OM432028, arrowed) aligned with closely related strains accessed from the GenBank. It showed 99.48% -100% identity and 99% - 100% coverage with several strains of *C. Lunata* including the type of strain, CBS 127235 with GenBank accession No. NR138223 (Fig. 2).

Isolates No. 80 was (*Fusarium solani* AUMC 15456 with GenBank accession No. OM432026, arrowed) aligned with closely related strains accessed from the GenBank. It showed 99.64% -99.82% identity and 99% - 100% coverage with several strains of *F. solani* including the type of strain, *F. solani* CBS 140079T with GenBank accession No. NR163531 (Fig. 3). *Acremonium egyptiacum* is identified as an outgroup strain on the phylogenetic tree. Pathogenicity tests and phylogenetic analyses of their geographic distribution are used in this study to characterise the pathogens that cause faba bean and leaf spot diseases.

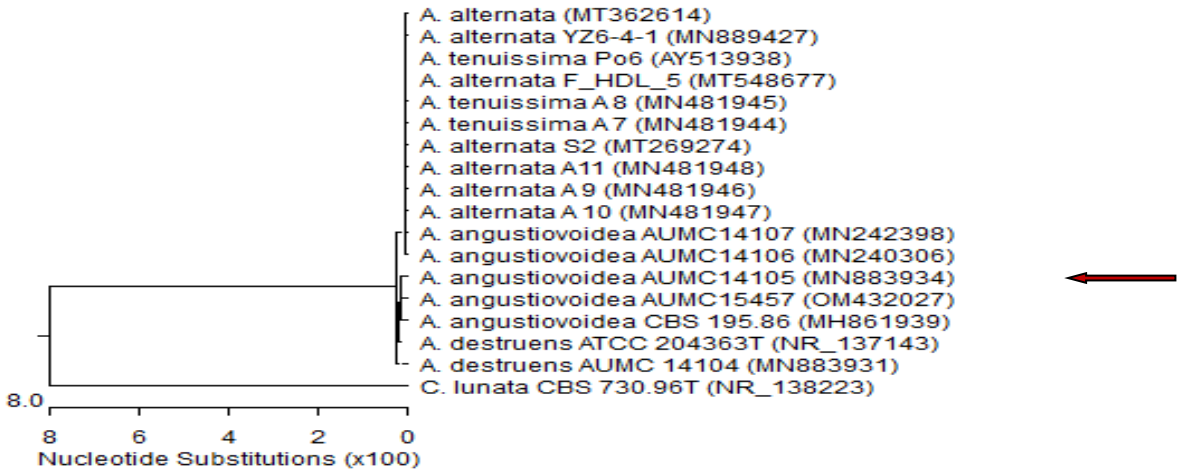

Fig 1. Maximum likelihood phylogenetic tree based its sequences and bootstrap support values. ITS sequences of rDNA of the isolated fungal strains (No.40) in the present study was *Alternaria angustiovoidea* (AUMC 15457) which was aligned with closely related sequences accessed from the GenBank.

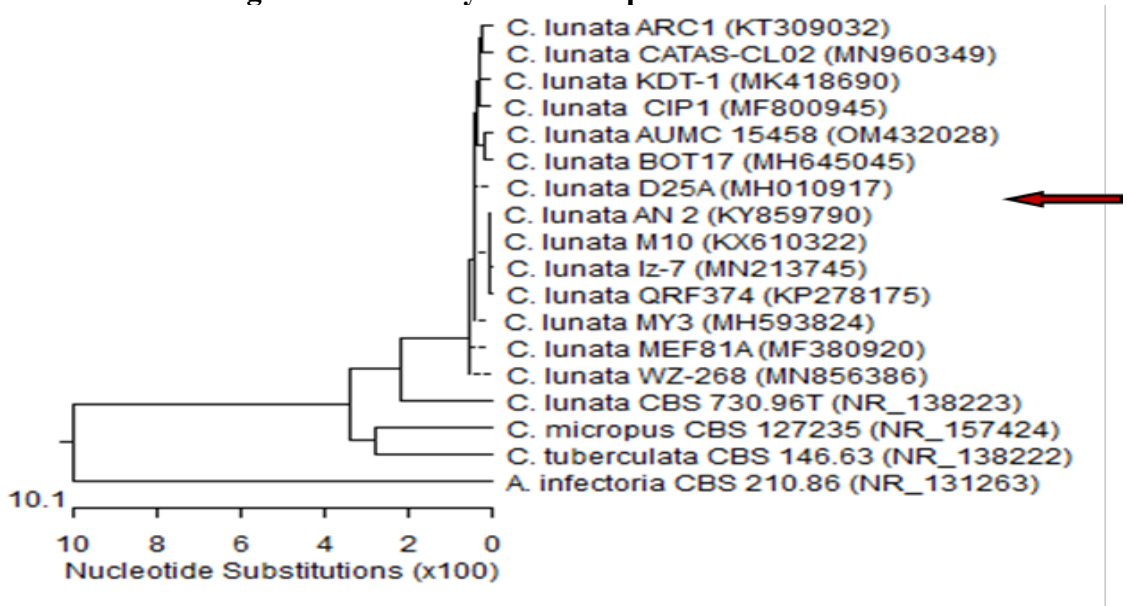

Fig (2): Maximum likelihood phylogenetic tree based its sequences and bootstrap support values. ITS sequences of rDNA of the isolated fungal strains (No.59) in the present study was *Curvularia lunata* (AUMC 15458) which was aligned with closely related sequences accessed from the GenBank.

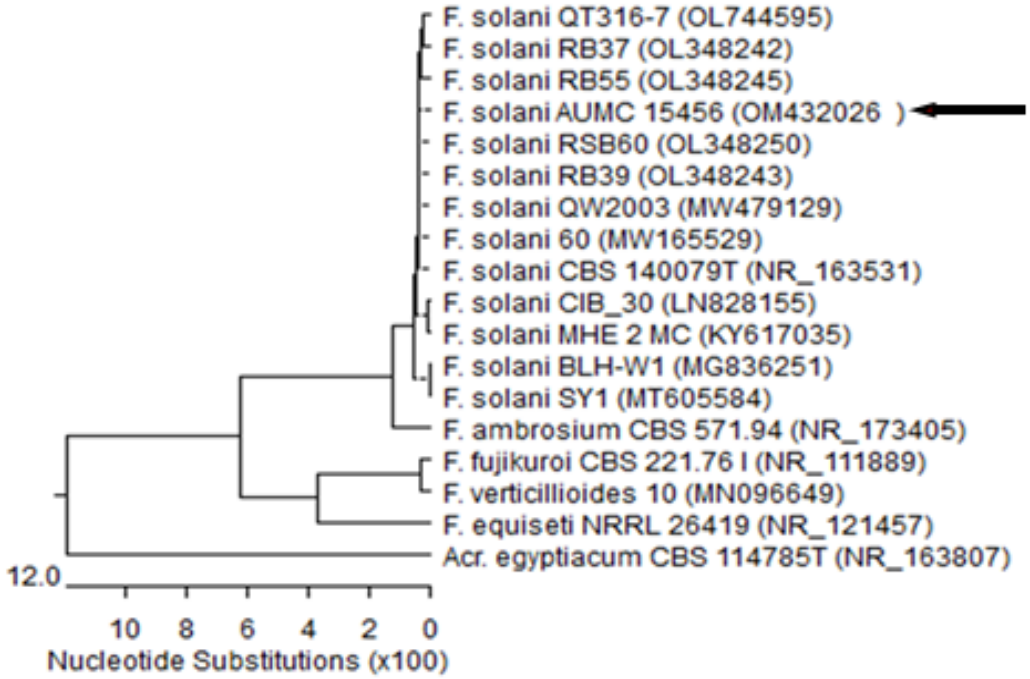

Fig 3. Maximum likelihood phylogenetic tree based its sequences and bootstrap support values. ITS sequences of rDNA of the isolated fungal strains (No.80) in the present study was *Fusarium solani* (AUMC 15456), which was aligned with closely related sequences accessed from the GenBank.

Reaction of certain cultivars to leaf spot disease under greenhouse conditions

Under greenhouse conditions in the 2020 growing season, the susceptibility of four faba bean cultivars (Giza 2, Giza 125 Giza 667, and Giza 843) to infection with the highly pathogenic isolates *Alternaria angustiovoidea*, *Curvularia lunata*, and *Fusarium solani*. The results presented in Table (2) and Figure (4) revealed that, there was a significant difference among cultivars in this response to leaf spot infection. In General, Giza 843 was the most susceptible cultivar to leaf spot infection with disease severity 64.51% as a mean to all three tested pathogenic isolates followed by Giza 125 with disease severity 48.78% while the highest tolerant cultivar to leaf spot disease was Giza 2 followed by Giza 667 as these two cultivar showed disease severity 22.91%, 34.58%, respectively as a mean of all three tested pathogenic isolates.

Alternaria angustiovoidea caused the highest disease severity in all tested cultivars followed by *Curvularia lunata* and *Fusarium solani*. The highest disease severity in faba bean occurred in case of Giza 843 infected with *Alternaria angustiovoidea* gave 88.97%, while the lowest ones was occurred in case of *Fusarium solani* with Giza 2 cultivar (5.97%).

Table 2: Reaction of certain four faba bean cultivars to infection with

Cultivar	Disease severity (%)			
	Alternaria angustiovoidea	***Curvularia lunata***	***Fusarium solani***	**Mean**
Giza 2	34.29^{ef*}	28.47^{f}	5.97^{g}	22.91^{e}
Giza 125	57.18^{b}	49.75^{c}	39.42^{de}	48.78^{b}
Giza 667	49.59^{c}	47.67^{c}	6.48^{g}	34.58^{c}
Giza 843	88.97^{a}	60.62^{b}	43.96^{cd}	64.51^{a}
Control	0.00^{g}	0.00^{g}	0.00^{g}	0.00^{g}
Total Mean	48.89^{a}	37.30^{b}	19.07^{c}	61.24^{a}

*Means in each column followed by the same letter are not significantly different according to Duncan's multiple range tests at 5%.

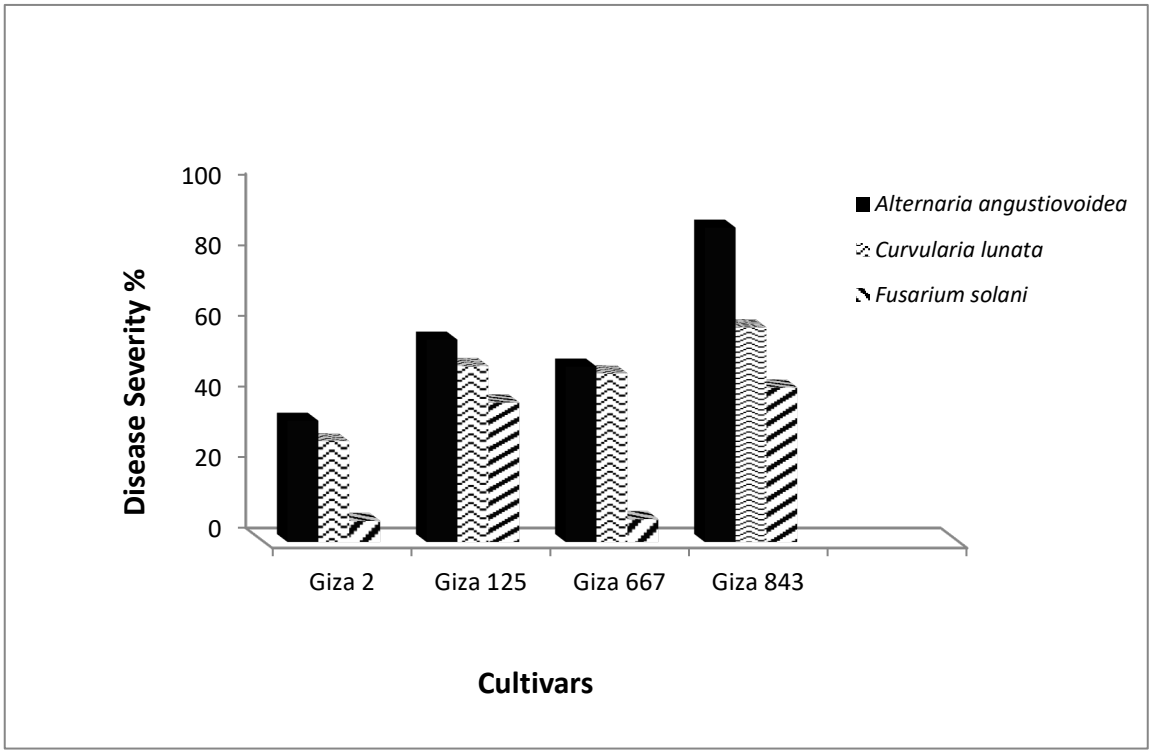

Fig 4. Reaction of four different faba bean cultivars to infection withthe causal pathogens of leaf spot disease.

Discussion

Understanding the fungi that cause faba bean leaf spot in Upper Egypt was the goal of this investigation. Eighty fungal isolates were isolated from different localities at Assiut and Sohag governorates. Pathogenicity varied greatly among the isolates was investigated. The high pathogenic isolates were *Alternaria angustiovoidea*, *Curvularia lunata* and *Fusarium solani*. These results are in line with those reported by Hassan, *et al*. (2022). *Alternaria angustiovoidea* and *Curvularia lunata* caused the highest disease severity, while *Fusarium solani* caused the lowest leaf spot severity %. Leaf spot caused by *Fusarium solani* on the leaves are circular, reddish brown and associated with discoloration to yellow of leaves these outcomes are consistent with Wehlburg, (1980) who showed that,

Fusarium moniliforme is the causal agent of Dracaena sp. and Pleomele sp. leaf spot and Mirhosseini *et al*., (2014) who mentioned that *Fusarium brachygibbosum* causing leaf spot on oleander in Iran, whereas *Curvularia lunata* isolate caused spot surround by yellow halo. These results support previous results that reported that, *Curvularia lunata* has main as a pathogen causing leaf spot of corn in United States (Garcia-Aroca *et al*., 2018). In this study *Curvularia lunata* and *Alternaria angustiovodea* caused lesions begin as little, brown circular spots on the lower leaves and gradually get larger before developing into concentric rings of brown with dark edges. These findings support those reported by El-Ammari (2017) and EL-Mougy *et al*. (2016).

Evaluation of certain faba bean cultivars to leaf spot reach showed that, these four faba bean cultivars varied in their response to faba bean infection the most resistant cultivars were Giza 2 followed by Giza 667 while the most susceptible cultivar was Giza 843 followed by Giza 125, this results in partial agreement with Behairy *et al*., (2014).

To summarize, fungal isolates from diseased faba bean plants from Assiut and Sohag Governorates (Upper Egypt) were tested for ability to cause leaf spot faba bean. Using PCR with ITS primers and sequencing of the PCR products. The three most pathogenic of these isolates were identified as *Alternaria angustiovodea, Curvularia luna*ta and *Fusarium solani*.

This is the first report of leaf spot caused by *Fusarium solani*, this pathogen may present a severe threat to faba bean production in Egypt. The management strategy should they adopted soon against this disease.

References

Booth, C. (1971). The genus *Fusarium*. Commonwealth Mycological Institute, Kew, Surrey, England, 237pp.

Behairy, Magda H.; Sobhy, H.M.; M.S. Abbas; Abada, K.H.A. and Mourad, M.Y. (2014). Alternaria leaf spot disease control on faba bean in Egypt. J. Plant Prot. and Path., Mansoura Univ., 5(1): 119-130.

Criscuolo, A. and Gribaldo, S. (2010). BMGE (Block Mapping and Gathering with Entropy): a new software for selection of phylogenetic informative regions from multiple sequence alignments. BMC Evolutionary Biology, 10: 210.

Dhingra, O.D. and Sinclair, J.B. (1985). Basic plant pathology Methodes. CRC, Press Inc., Boca Raton Florida USA.353pp.

EI-Ammari, A.S. (2017). Plant Fungal Diseases of Faba bean in Benghazi, Department of Plant Production, Faculty of Agriculture, University of Benghazi, 1-5.

El-hendawy, S.; Shaban, W. and Sakagami, J.I. (2010). Does treating faba bean seeds with chemical inducers simultaneously increase chocolate spot disease resistance and yield under fieldconditions. Turk. J. Agric., 34: 475-485.

Ellis, M. (1976). More dematiaceous hyphomycetes. Kew, Surrey, England: Commonwealth Mycological Institute, 507pp.

El-Mougy, S.N.; Abdel-Kader, M.M.; Shabn, A.M. and Abdel-Aziz, A. (2016). Broad bean a new host of leaf spot disease caused by *Alternaria tenuissima* in North Egypt. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 7(3): 571-579.

Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. Evolution, 39(4): 783-791.

Garcia-Aroca, T.; Dolye, V. and Singh, R. (2018). First Report of Curvularia Leaf spot of Corn, Caused by *Curvularia Lunata*, in the United States. Plant Health Progress, 19:140-142.

Gomez, K.A. and Gomez, A.A. (1984). Statically procedures for Agricultural Research. 2nd Edition, John Wiley and Sons Inc., New York, USA., 680pp.

Guindon, S.; Dufayard, J.F.; Lefort V.; Anisimova, M.; Hordijk, W. and Gascuel, O. (2010). New Algorithms and Methods to Estimate Maximum-Likelihood Phylogenies: Assessing the Performance of PhyML 3.0. Systematic Biology, 59(3): 307–321.

Hassan, Naglaa, M.S.; Abd El-Aal, A.; Hassan, M. and Khalaphallah, R. (2022). Isolation, identification and biocontrol treatments of *Alternaria Alternata* of *Vicia faba.* SVU-International Journal of Agricultural Sciences, 4(4): 158-167.

Hawatin, G. and Webb, C. (1982). Faba bean improvement. (eds) Aleppo: ICARDA. 191-198 pp.

Juroszek, P. and von Tiedemann, A. (2011). Potential strategies and future requirements for plant disease management under a change climate. Plant Pathology, 60(1): 100-112.

Katoh, K. and Standley, D.M. (2013). MAFFT multiple sequence alignment software version7: improvements in performance and usability. Molecular biology and evolution, 30(4):772-780.

Lefort, V.; Longueville, J.E. and Gascuel, O. (2017). SMS: Smart model selection in PhyML. Molecular biology and evolution, 34(9): 2422-2424.

Mahmoud, N.M.A. (1996). Studies on chocolate spot disease of broad bean and loss occurrence. Ph.D. Thesis, Fac. Agric., Minufiya Univ., 133pp.

Mirhosseini, H.A.; Babaeizad, V. and Hashemi, L. (2014). First report of *Fusarium brachygibbosum* causing leaf spot on Oleander in Iran. Journal of Plant Pathology, 96(2): 431-439.

Pitt, J.I. and Hocking, A.D. (2009). Fungi and food spoilage 3rd Ed Springer Science and Business Media, London New York.

Rahman, M.Z.; Honda, Y.; Islam, S.Z.; Muroguchi, N. and Arase, S. (2002). Leaf Spot Disease of Broad Bean (*Vicia faba* L.) caused by *Alternaria tenuissima*-A New Disease in Japan. J. Gen. Plant Pathol., 68: 31–37.

Reis, R.F.; de Almeida, T.F.; Stuchi, E.S. and de Goes, A. (2007). Susceptibility of citrus species to *Alternaria alternata* the causal agent of the Alternaria brown spot. Scientia Horticulturae, 113(4): 336-342.

Sahile, S.; Fininsa, C.; Sakhuja, P.K. and Ahmed, S. (2008). Effect of mixed cropping and fungicides on chocolate spot (*Botrytis fabae*) of faba bean (*Vicia faba*) in Ethiopia. Crop Protection, 27(2): 275-282.

Simmons, E.G. (2007). *Alternaria:* An Identification Manual.

Wehlburg, C. (1980). Leaf spot of *Dracaena marginatalam* caused by *Fusarium moniliforme Sheld*. Proc. Fla. Sate Hort. Soc., 80: 454-456.

White, T.J.; Bruns, T.; Lee, S. and Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In PCR Protocols: A guide to Methods and Applications (ed. M.A. Innis, D.H. Gelfand, J.J. Sninsky and T.J. White), pp. 315-322. Academic Press: San Diego, U.S.A.

التعريف الجزيئي لمسببات مرض تبقع أوراق الفول في صعيد مصر

هند شحاتة عبد الراضي شحاته*[1]، قناوي محمد حسن عبد المنعم[1]، محسن السيد على ابورحاب[2]، امال محمد ابراهيم عراقى[1]

[1]قسم امراض النبات، كليه الزراعة، جامعه أسيوط، مصر
[2]معهد بحوث امراض النبات، مركز البحوث الزراعية، الجيزة، مصر

الملخص

يعتبر الفول البلدي أهم محصول بقولي في مصر. تم الحصول على ثمانين عزلة فطرية تم عزلها من أوراق الفول البلدي التي تظهر عليها اعراض مرض تبقع الأوراق والمنزرعة في مواقع مختلفة بمحافظتي أسيوط وسوهاج. تم تعريف جميع العزلات باستخدام الخصائص المورفولوجية لخصائص الميسليوم والجراثيم. تم تعريف ثلاث عزلات التي أظهرت أعلى قدرة مرضية بالطرق الجزيئية باستخدام تسلسل ITS-rDNA (ITS1 وITS4). بناءً على نتيجة تسلسل ITS، تم تعريف العزلات الفطرية وهي (OM432027) *Alternaria angustiovoidea*، (OM432028) *Curvularia lunata*، (OM432026) *Fusarium solani*. تم إجراء اختبارات القدرة المرضية في الصوبة المفتوحة على صنف الفول جيزة 843، حيث كانت النسبة المئوية لشدة الإصابة هي 62.23٪ في حالة الإصابة بالفطر *A. angustiovoidea* وكانت62.66% في حالة الإصابة بالفطر *Curvularia lunata* بينما كانت النسبة المئوية لشدة الإصابة مع العدوى بالفطر*F. Solani* حوالي 50.23 % أظهر تقييم تفاعل أربعة أصناف من الفول البلدي لمرض تبقع الأوراق أن الصنف جيزة 843 كان الأكثر حساسية يليه جيزة 125 بينما كان الصنف جيزة 2 أكثر الأصناف تحملاً يليه الصنف جيزة 667.