Original Article

Endophytic fungi associated with chickpea (*Cicer arietinum* L.) in Iran

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ABSTRACT

Chickpea (*Cicer arietinum*) is an important legume crop. This annual plant is widely cultivated across many regions of the world. A notable characteristic of chickpeas is their multiple symbiotic associations with diverse microorganisms that contribute to soil fertility. In this study, we investigated the diversity of endophytic fungi in seeds, aerial and underground organs of twenty-one genotypes of chickpea. The genotypes were obtained from the Dryland Agricultural Research Institute (Maragheh, East Azerbaijan, Iran). The genotypes were grown under both greenhouse and field conditions using disinfected and non-disinfected seeds. Additionally, to assess endophytic fungal diversity under field conditions, sampling was conducted in chickpea fields in the villages of Anjirak and Znylaan-Sofla, Kermanshah County, as well as in fields on the experimental campus of the Faculty of Agriculture, University of Razi, Kermanshah, Iran. A total of 366 fungal strains were isolated, of which 80 isolates were obtained from plants grown from non-disinfected and 60 isolates from disinfected seeds in the greenhouse. Moreover, from chickpea genotypes planted on a farm in Hamadan Province and 48 plant samples collected across six fields in Kermanshah Province, a total of 50 and 155 endophytic fungal isolates were obtained, respectively. Furthermore, a total of 21 fungal isolates were recovered from seeds of genotypes 'Desi 37', 'Kaka', 'Sufi', and 'Flip 09-2780'. Following morphological characterization and sequencing of representative isolates based on the genomic regions *tef1-α*, *tub2*, ITS rDNA, *cal* and *rpb2*, ten fungal species were identified, belonging to six genera. The identified species were *Allophoma labilis, Aspergillus fumigatus, A. luchuensis, A. niger, A. tubingensis, Chaetomium rectangulare*, *Cladosporium ramotenellum, Fusarium acuminatum, F. redolens* and *Penicillium chrysogenum.* The most isolates belong to *Aspergillus* (60.65%) and *Fusarium* species (27.86%). Among the chickpea genotypes, the greatest number of isolates were obtained from 'Flip 09-2780' (43 isolates) and 'Adel' (41 isolates).

KEYWORDS

Aspergillus, *Fusarium*, fungal biodiversity, legume, mutualistic relationship.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the most important legume crops after beans and peas. Globally, approximately 2.3 million tons of chickpeas are imported annually, and the crop is cultivated in more than 40 countries (Merga and Haji 2019). Iran, Syria and Turkey are the largest producers of chickpea in western Asia, accounting for 16% of global production (Ahmad et al. 2005). Chickpea contributes significantly to supplying 20-25% of human protein requirements and is considered one of the cheapest protein sources (Grasso et al. 2022). It also has a profound impact on agricultural ecology, particularly soil fertility, due to its mutualistic relationship with various microorganisms (Joshi and Rao 2017). Endophytes are prominent among these

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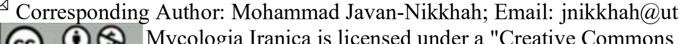
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microorganisms. Endophytes are microorganisms that colonize the internal tissues of healthy host plants for all or part of their life cycle without causing damage at that time (Wani et al. 2015). Endophytes are also important in the agricultural industry due to their production of secondary metabolites and their enhancement of plant resistance to biotic and abiotic stresses (Watts et al. 2023).

Given global population growth, various strategies are recommended to reduce stress and improve plant efficiency, such as the use of fertilizers. However, some fertilizers may be ineffective for these purposes and could contribute to environmental pollution; moreover, they may be associated with an increased cancer risk in humans (Nazarideljou et al. 2024). Hence, innovative and eco-friendly approaches, such as endophytes that promote plant growth through the production of indole-3-acetic acid (IAA), siderophores, and other bioactive compounds have attracted attention (Singh et al. 2018).

Numerous studies have identified endophytes in various crops, such as soybean (Kim et al. 2023) and rice (Gong et al. 2025). In other studies, attempts have been made to assess the potential of endophytes for controlling plant pathogens. For instance, García-Latorre et al. (2025) reported that the endophytic fungus, *Purpureocillium lilacinum* had a significant impact on *Botrytis cinerea* in chickpea. Moreover, Bazghaleh et al. (2015) isolated *Trichoderma harzianum* and *Mortierella alpina* as endophyte from chickpea roots. Meghana et al. (2022) obtained some endophytic fungi from roots, stems and chickpea leaves belonging to genera such as *Aspergillus*, *Fusarium* and *Alternaria*.

To date, no endophytic fungi have been reported in chickpea plants in Iran. This study aimed to identify endophytic fungi associated with different organs across a range of Iranian chickpea genotypes.

MATERIALS AND METHODS

Fungal isolates

Fungal isolates were obtained from (a) seeds of 21 chickpea genotypes, (b) plants of these genotypes grown under greenhouse and field conditions, and (c) plants of commonly cultivated genotypes from farms across three regions in Kermanshah County, Iran (villages of Anjirak and Znylaan-Sofla, as well as in fields on the experimental campus of the Faculty of Agriculture, University of Razi).

Seeds

Twenty-one chickpea genotypes, belonging to the Desi and Kabuli types (Table 1), were obtained from the Dryland Agricultural Research Institute (Maragheh, East Azerbaijan Province, Iran). After rinsing the samples under running tap water, the seeds were surface sterilized by sequential immersion in 95% ethanol for 1 min, sterile distilled water for 1 min, and a 3.5% sodium hypochlorite solution with Tween 20 (1 drop per 10 mL sodium hypochlorite) for 2 minutes. The seeds were then rinsed three times with sterile distilled water. After surface dehydration with sterile paper towels, each seed was crushed into small segments (Kumari et al. 2018) and the segments were placed in Petri dishes containing 2% potato dextrose agar (Larran et al. 2007) amended with 100 mg l^{-1} of antibiotic (Chloramphenicol). Plates were incubated in darkness at 25 °C.

Plants grown in greenhouse and field

To isolate endophytic fungi associated with both underground and aerial organs from 21 chickpea genotypes (Table 1), three seeds per genotype were selected and sown under two conditions in a greenhouse: with and without disinfection. Seed disinfection prior to sowing involved sequential immersion in: (i) 0.1% Triton X-100 emulsion for 2 minutes, (ii) 0.5% sodium hypochlorite for 2 minutes, and (iii) 70% ethanol for 2 minutes, with three successive rinses in sterile distilled water between steps. Following surface drying on sterile paper towels, disinfected seeds were placed on potato dextrose agar (PDA) plates and incubated at 25°C in the dark to assess germination (Parsa et al. 2016). Germinated seeds were then individually sown in plant trays (8×8 cm) containing sterilized vermiculite substrate, which had been autoclaved three times. Non-disinfected seeds were sown in sterilized vermiculite substrates in separate trays. To compare endophytic fungal diversity between genotypes under greenhouse and natural conditions, each genotype cultivated in the greenhouse was additionally planted in a field located in Tuyserkan (Hamedan Province, Iran) at a depth of 7 cm, with two replications in April 2024. Finally, chickpeas grown under both greenhouse and field conditions were sampled at the 7-8 leaf stage.

Table 1. Twenty-one chickpea genotypes (Desi and Kabuli types) used in this study were obtained from Dryland Agricultural Research Institute, Iran.

Type	Genotype
Desi	‘Moghadamati-desi17’
	‘Moghadamati-desi23’
	‘Moghadamati-desi53’
	‘Desi24’
	‘Desi19’
	‘Desi50’
	‘Desi37’
	‘Desi25’
	‘Kaka’
Kabuli	‘Sufi’
	‘Adel’
	‘Moghadamati-paiizeh’
	‘Line3’
	‘Flip09-2780’
	‘Azad’
	‘Barekat’
	‘Pishrafteh-paiizeh Mashhad’
	‘Moghadamati-bahareh98’
	‘Anna’
	‘Sazegari-bahareh Maragheh’
	‘Saiid’

Table 2. Morphological information of isolates obtained in this study.

Genus	Respective media	Abbreviation	Microscopic structures	Examined features	Identification reference
Chaetomium	Oatmeal Agar	OA	Ascocarp	color, germ pores, shape, size	Abdel-Azeem (2019)
	Czapek Yeast Agar	CYA	Ascomata hairs	color, length, and structure of terminal hairs	
	Malt Extract Agar	MEA	Asci	not observed	
			Ascospores	color, shape, size	
Fusarium	Potato Dextrose Agar	PDA	Sporodochia	Color	Han et al. (2023)
	Carnation Leaf Agar	CLA	Macroconidia	shape, shape of dorsal and ventral sides, shape of apical and basal cells, number of septa, size	
	Synthetic Nutrient Deficient Agar	SNA	Microconidia	shape, number of septa, arrangement on phialid (false head), size	
			Conidiogenous cell	Structure (monophialide, polyphialide)	
			Chlamydospore	Structure (chain or solitary), size	
Aspergillus	Czapek Yeast Agar	CYA	Conidial head	Structure (uniseriate or biseriate), size	Varga et al. (2011) Samson et al. (2007)
	Malt Extract Agar	MEA	Vesicles	Shape, diameter, and covering by phialides	
			phialides	size	
			Conidia	Shape, texture, size	
Penicillium	Czapek Yeast Agar	CYA	Phialides	Arrangement on metulae, size	Visagie et al. (2014)
	Malt Extract Agar	MEA	conidiophore	Size, type of branching (Monoverticillate, Biverticillate, Terverticillate)	
			conidia	Shape, texture, size	
Cladosporium	Potato Dextrose Agar	PDA	Mycellium	Width, branched or unbranched	Bensch et al. (2018)
			Conidiophores	Shape, color, number of septa, head-like swollen apices, texture, width, arrangement (solitary or chain), morphological differentiation from vegetative hyphae (micronematous, macronematous, semi-macronematous)	
	Synthetic Nutrient Deficient Agar	SNA	Conidia	shape, size, number of septa, arrangement in chain (branched or unbranched)	
			Ramoconidia	Shape, color, number of septa, size	
			Secondary ramoconidia	Shape, number of septa, size	
Allophoma	Potato Dextrose Agar	PDA	Pycnidium	Shape, color, diameter	
	Oatmeal Agar	OA			
			conidia	Color, shape, size	Chen et al. (2015)
	Malt Extract Agar	MEA			

Genotypes commonly cultivated on various farms

Samples were collected from the common chickpea genotypes grown by farmers across three areas in Kermanshah County, Kermanshah Province, a major hub for chickpea cultivation in Iran. In June 2023, healthy chickpea plants were randomly sampled from six farms located in the Anjirak and Zinlan Sofla villages under dryland conditions, as well as from the Faculty of Agriculture's research farms at Razi University (Kermanshah, Iran) under irrigated conditions. A total of 48 samples were collected.

Endophytic fungi were isolated from chickpea plant samples collected from field and greenhouse conditions, as well as from various farms. Each collected sample was transported to the laboratory and processed within 24–48 hours.

Sample preparation for fungal isolation

Each organ (underground and aerial) was cut into small segments with sterilized scissors and rinsed under running tap water for 15 min. Leaves, roots, and pods were surface sterilized by sequential immersion in: (i) 1% sodium hypochlorite for 1 min, followed by three rinses in sterile distilled water, (ii) 70% ethanol for 10 seconds, followed by three additional rinses in sterile distilled water (Meghana et al. 2022). Stems were surface sterilized by sequential immersion in 4% sodium hypochlorite for 2 min, followed by three rinses in sterile distilled water (Chen et al. 2020). After surface drying on sterile paper towels, each disinfected organ was placed in Petri dishes containing 2% potato dextrose agar (Larran et al. 2007) amended with 100 mg l^{-1} of antibiotic (Chloramphenicol). Plates were incubated in

Table 4. Primers used for DNA amplification and sequencing of fungal isolates.

Locus	Primer	Direction	Primer sequence (5ˈ-3ˈ)	PCR conditions	Reference
Internal Transcribed Spacer (ITS) of rDNA	ITS1	Forward	TCCGTAGGTGAACCTGCG	95°C 5 min; 35 cycles of 94°C 45s; 57°C 45s; 72°C 45s;72°C 7 min	White et al. (1990)
	ITS4	Reverse	TCCTCCGCTTATTGATAC		White et al. (1990)
RNA polymerase II second-largest subunit (*rpb2*)	rpb2-5F2	Forward	GGGGWGAYCAGAAGAC	95°C 5 min; 40 cycles of 95°C 60s; 55°C 2 min; 72°C 90s;72°C 10 min	Sung et al. (2007)
	rpb2-7CR	Reverse	CCCATRGCTTGYTTRCCT		Liu et al. (1999)
Translation elongation factor1-alpha (*tef1-α*)	EF-1	Forward	ATGGGTAAGGARGACAC	95°C 2 min; 35 cycles of 95°C 30s; 55°C 30s; 72°C 2 min;72°C 10 min	O'Donnell et al. (1998)
	EF-2	Reverse	GGARGTACCAGTSATCAG		O'Donnell et al. (1998)
	EF1-728	Forward	CATCGAGAAGTTCGAGG	94°C 5min; 40 cycles of 94°C 45s; 52°C 30s; 72°C 90s;72°C 6 min	Carbone and Kohn (1999)
	EF1-986	Reverse	TACTTGAAGGAACCCTTC		Carbone and Kohn (1999)
Calmodulin (*cal*)	CMD5	Forward	CCGAGTACAAGGARGCC	95°C 10 min; 35 cycles of 95°C 1 min; 55°C 1 min; 72°C 1 min;72°C 10 min	Hong et al. (2005)
	MD6	Reverse	CCGATRGAGGTCATRAG		Hong et al. (2005)
β-tubulin (*tub2*)	Bt2a	Forward	GGTAACCAAATCGGTGC	94°C 5 min; 40 cycles of 94°C 45s; 55°C 45s; 72°C 60s;72°C 7 min	Glass and Donaldson (1995)
	Bt2b	Reverse	ACCCTCAGTGTAGTGACC		Glass and Donaldson (1995)
	T1	Forward	AACATGCGTGAGATTGT	94°C 10 min; 40 cycles of 94°C 30s; 57°C 30s; 72°C 30s;72°C 10 min	O'Donnell and Cigelnik (1997)
	TUB4Rd	Reverse	CCRGAYTGRCCRAARARA AGTTG TC		Woudenberg et al. (2009)

darkness at 25 °C and fungal growth was observed.

Morphological identification

After purification using the hyphal tipping method, isolates were cultured on their respective media (Table 2) to assess colony characteristics, including growth rate, surface and reverse colony coloration, and colony texture. Fungal structures were mounted in lactophenol cotton blue for microscopic analysis. Measurements were taken at 30 points (×100 magnification) from the structures described in Table 2, to capture morphological variation.

PCR amplification

A representative set of isolates (n = 12) based on distinctive morphological features (Table 2) was selected for molecular analysis. Genomic DNA was extracted from fresh, purified mycelia grown on PDA at 25°C for 7–10 days, following the method described by Zhong and Steffenson (2001). DNA was stored at −20°C in 1.5 mL tubes until use. The corresponding genomic loci were amplified by PCR using primer pairs described in Table 3. Each PCR reaction (25 µL total volume) contained: 10 µL PCR Master Mix (Tehran Cavosh Clon/Sina Clon, Tehran, Iran), 11 µL nuclease-free water, 1 µL forward primer (10 µM), 1 µL reverse primer (10 µM), and 2 µL template DNA. Table 3 lists the PCR conditions for each genomic locus. PCR products were visualized on 1% agarose gels. Successful amplicons were sequenced by the Codon Genetic Company (Tehran, Iran).

Phylogenetic analyses

Sequences obtained in this study, along with acquired GenBank sequences, and sequences from out group's species were aligned using MAFFT v7 (online server). Misalignments were manually corrected in BioEdit v7.0.0 (Hall, 2004). When necessary, individual gene alignments were concatenated using Mesquite v2.75 to generate a combined dataset for phylogenetic analyses. Phylogenies were inferred under three complementary algorithms: Maximum Likelihood (ML), Maximum Parsimony (MP), and Bayesian Inference (BI). Maximum Likelihood analyses were performed via the CIPRES Science Gateway utilizing RAxML-HPC2 on ACCESS v. 8.2.12 and IQ-TREE for model selection and ML inference as appropriate. Bayesian Inference and MP analyses were conducted via the CIPRES Science Gateway. Bayesian Inference employed MrBayes (accession v3.2.7a) and MP utilized PAUP (access on XSEDE, v4.0a168). ModelFinder within IQ-TREE was used to identify the best-fitting evolutionary models. Across all phylogenetic trees, support values were interpreted as ML and MP bootstrap values > 50% and BI posterior probabilities > 0.80. Generated sequences were submitted to GenBank. Typified isolates were deposited in the culture collection (IRAN) at the Iranian Research Institute of Plant Protection (Tehran, Iran). Accession numbers and collection identifiers are listed in Supplementary Table 1.

RESULTS

Sampling and fungal isolation

From the seeds of four genotypes (Desi 37, Kaka, Sufi, and Flip 09-2780), 21 fungal isolates were recovered, belonging to *Aspergillus* (57.14%) and *Cladosporium* (42.85%). The genotypes 'Desi 37' and 'Flip 09-2780' each yielded the highest number of isolates (seven isolates each). In a greenhouse experiment, we employed two sowing treatments (with and without seed disinfection) using seeds from various chickpea genotypes (Table 1). We obtained 80 fungal isolates from plants grown from non-disinfected seeds and 60 isolates from plants grown from disinfected seeds. *Aspergillus* spp. were the dominant species, representing 100% of isolates from non-disinfected seeds and 91.66% from disinfected seeds. Remaining isolates from disinfected seeds belong to *Fusarium* spp. (8.34%). The plant roots, stems and leaves of non-disinfected seeds accounted for 30%, 47.50% and 22.5% of isolates, respectively, while the plant roots, stems and leaves of disinfected seeds accounted for 45%, 41.66% and 13.33%, respectively. The 'Kaka' genotype (non-disinfected seeds) contributed 26.25% of isolates, and the 'Flip 09-2780' genotype (disinfected seeds) contributed 31.66% of isolates, representing the largest shares in their respective treatments.

From chickpea genotypes (Table 1) planted on a farm in Hamadan Province, 50 endophytic fungal isolates were obtained. *Aspergillus* spp. was identified as the dominant genus and accounted for 66% of the isolates. By contrast, *Penicillium* spp., *Cladosporium* spp., *Chaetomium* spp. and *Fusarium* spp. experienced the contribution of 8%, 16%, 2% and 8% respectively. Among the genotypes tested under field conditions, 'Adel' and 'Moghadamati-paiizeh' yielded the highest number of isolates, each accounting for 30% of the total. The majority of endophytic fungi (50%) were recovered from leaves and stems, whereas no isolates were obtained from the roots.

A total of 155 fungal isolates were obtained from 48 plant samples collected from six fields in Kermanshah Province. *Fusarium* spp. (60%) and *Aspergillus* spp. (27.09%) were the dominant genera. Other recovered genera included *Chaetomium* sp. (4.51%), *Cladosporium* sp. (7.74%), and *Allophoma* sp. (0.64%). The distribution of isolates across plant tissues varied considerably between irrigated and dryland conditions. Under irrigated conditions, the roots harbored the highest proportion of isolates (9.67%), followed by leaves (6.45%) and stems (0.64%); no isolates were

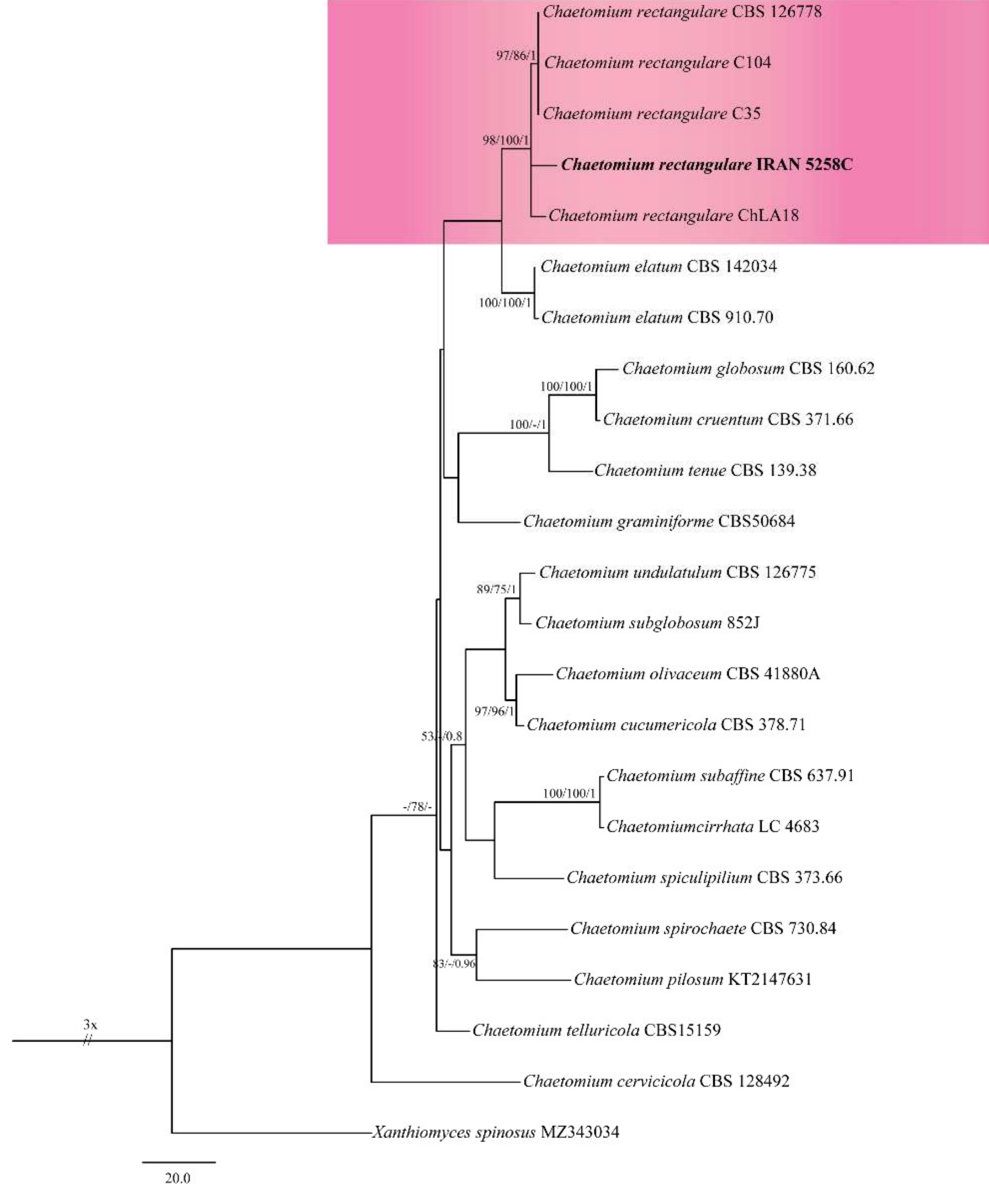

Fig. 1. One of the three equally most parsimonious phylogenetic trees inferred from the *tub2* sequence data across *Chaetomium* species. The tree is rooted to *Xanthiomyces spinosus* MZ343034. The scale bar indicates the number of nucleotide substitutions per site. Bootstrap support values from Maximum Likelihood (ML) and Maximum Parsimony (MP), along with posterior probabilities from Bayesian Inference (BI), are shown as ML-BS / MP-BS /BI-PP at the nodes.

recovered from pods. In contrast, under dryland conditions, the roots again yielded the most isolates (43.87%), followed by stems (25.80%), pods (9.03%), and leaves (4.51%).

Fungal species

In this study, 10 fungal species belonging to six genera (*Chaetomium*, *Fusarium*, *Allophoma, Cladosporium*, *Aspergillus* and *Penicillium*) were identified following morphological characterization and molecular analysis.

Chaetomium rectangulare Asgari & Zare, Mycologia 103 (4): 872 (2011).

The taxonomic position of the *Chaetomium* sp. isolate IRAN 5258C was determined using *tub2*.

Alignment of the dataset comprised 676 characters (including gaps), of which 410 were constant; 133 were parsimony-uninformative or parsimony-informative. Maximum parsimony (MP) analysis of the remaining 133 parsimony-informative characters led to three parsimonious trees (TL=465, CI=0.742, RI=0.691, HI=0.258). IQ-TREE best tree (log-likelihood -3180.069) was determined after iterations. The best-fitting evolutionary model picked out by Model Finder in IQ-TREE was HKY+F+G4. The Bayesian analyses of the *tub2* locus formed 1102 trees, of which 274 were eliminated as burn-in. The consensus tree and posterior probability values (PP) were calculated from the remaining 828 trees. The average standard deviation of split frequencies at the end of the run was 0.008273. Based on phylogenetic analyses, isolate IRAN 5258 clustered in a well-supported clade with *Chaetomium rectangulare* strain ChL-A18 (Fig.1). A total of 14 isolates of *Chaetomium* spp. were obtained in this study, of which eight were identified as *C. rectangulare* from the leaves of two healthy asymptotic genotypes ('Bivanij', and 'Kaka'). These isolates originated from two geographic regions, specifically, Zinlansofla and Toyserkan, in Kermanshah and Hamadan Provinces, Iran.

Note: This species has previously been reported from wheat in Iran (Asgari and Zare 2011).

Fusarium* spp.** (including ***Fusarium acuminatum Ellis & Everh., Proc. Acad. Nat. Sci. Philadelphia 47: 441 (1895); ***Fusarium redolens*** Wollenw., Phytopathology 3 (1): 29) (1913).

The taxonomic position of the representative *Fusarium* spp. isolates (IRAN 5259C, IRAN 5260C) was determined using *tef1-α*. Following alignment, the dataset comprised 708 characters, including alignment gaps, of which 372 were constant, 99 were variable and parsimony-uninformative, and 237 were parsimony-informative. Maximum parsimony (MP) analysis of the 237 parsimony-informative characters yielded four parsimonious trees (TL=761, CI=0.681, RI=0.811, HI=0.319). The IQ-TREE best tree (log-likelihood = -4459.579) was inferred after iterations. The best evolutionary model picked out by Model Finder in IQ-TREE was TIM2+F+I+G4. The Bayesian analyses of the *tef1-α* locus produced 5202 trees, of which 1,300 were eliminated as burn-in. The consensus tree and posterior probability values (PP) were derived from the remaining 3902 trees. The average standard deviation of split frequencies at the end of the run was 0.009453. Based on phylogenetic analyses IRAN 5259C and IRAN isolates clustered in a well-supported clade containing *Fusarium acuminatum* strain NRRL54214 and *Fusarium redolens* strain MT 409452 (Fig.2). A total of 97 Fusarium isolates were recovered. Among these, 30 isolates were identified as *F. acuminatum*, obtained from healthy leaves and roots of the 'Bivanij' and 'Adel' genotypes. The remaining 67 isolates were identified as F. redolens, which were exclusively isolated from healthy roots of the 'Bivanij' genotype. These F. redolens isolates originated from three distinct geographical areas in Kermanshah Province, Iran: Znylaan Sofla, Anjirak, and the research farms of the Faculty of Agriculture at Razi University.

Note: *Fusarium acuminatum* has been identified as a pathogen infecting chickpea roots and seeds in Montana, USA (Moparthi et al. 2024). In contrast, reports on *F. redolens* present a dual ecological role. While it has been documented as an endophyte in various plants, including rice, olive, and barley, it has also been reported as an important pathogen in chickpea (Jamali 2024).

Allophoma labilis (Sacc.) Qian Chen & L. Cai, Stud. Mycol. 82: 162 (2015).

The taxonomic position of the *Allophoma* isolate (IRAN 5261C) was determined using the *rpb2* gene. The dataset comprised 758 characters after alignment, including gaps. Among these, 565 characters were constant, 61 were variable but parsimony-uninformative, and 132 were parsimony-informative. Maximum parsimony (MP) analysis of the 132 parsimony-informative characters yielded three parsimonious trees (TL=369, CI=0.653, RI=0.678, HI=0.347). IQ-TREE best tree (log-likelihood = -2747.409) was obtained after iterations. The best evolutionary model selected by Model Finder in IQ-TREE was TNe+G4. Bayesian analyses of the *rpb2* locus yielded 402 trees, of which 100 were eliminated as burn-in. The consensus tree and posterior probability values (PP) were calculated from the remaining 302 trees. The average standard deviation of split frequencies at the end of the run was 0.006244. Based on phylogenetic analyses, isolate IRAN 5261C clustered in a well-supported clade with *Allophoma labilis* strain CBS 124.93 (Fig. 3). A total of one isolate of *Allophoma* sp. was obtained from the stem of 'Adel' genotype of healthy *Cicer arietinum* plants showing no disease symptoms, collected from a single geographical area located in Kermanshah Province, Iran—the research farms of the Faculty of Agriculture at Razi University.

Note: Larki et al. (2019) reported *Allophoma labilis* as a pathogen in *Catharanthus roseus* in Iran. Furthermore, this species was identified as a pathogen of tomato blight in Brazil (Colman et al. 2018).

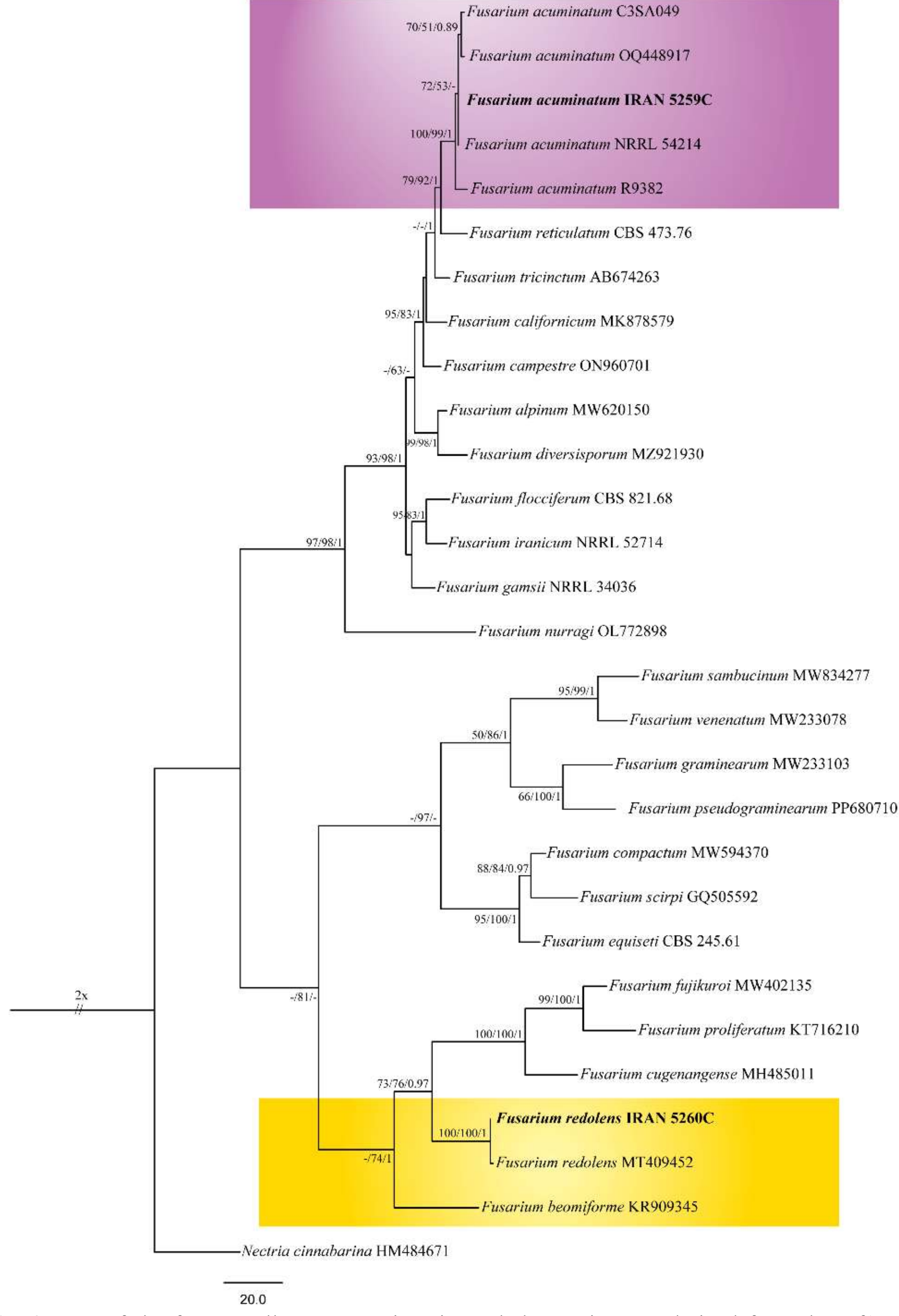

Fig. 2. One of the four equally most parsimonious phylogenetic trees derived from the tef1-α genomic locus sequence data across *Fusarium* species. The tree was rooted to *Nectria cinnabarina* HM484671. The scale bar indicates the estimated number of nucleotide substitutions per site. Bootstrap support values obtained from Maximum Likelihood (ML) and Maximum Parsimony (MP), along with posterior probabilities from Bayesian Inference (BI), are presented at the nodes as ML-BS/MP-BS/BI-PP.

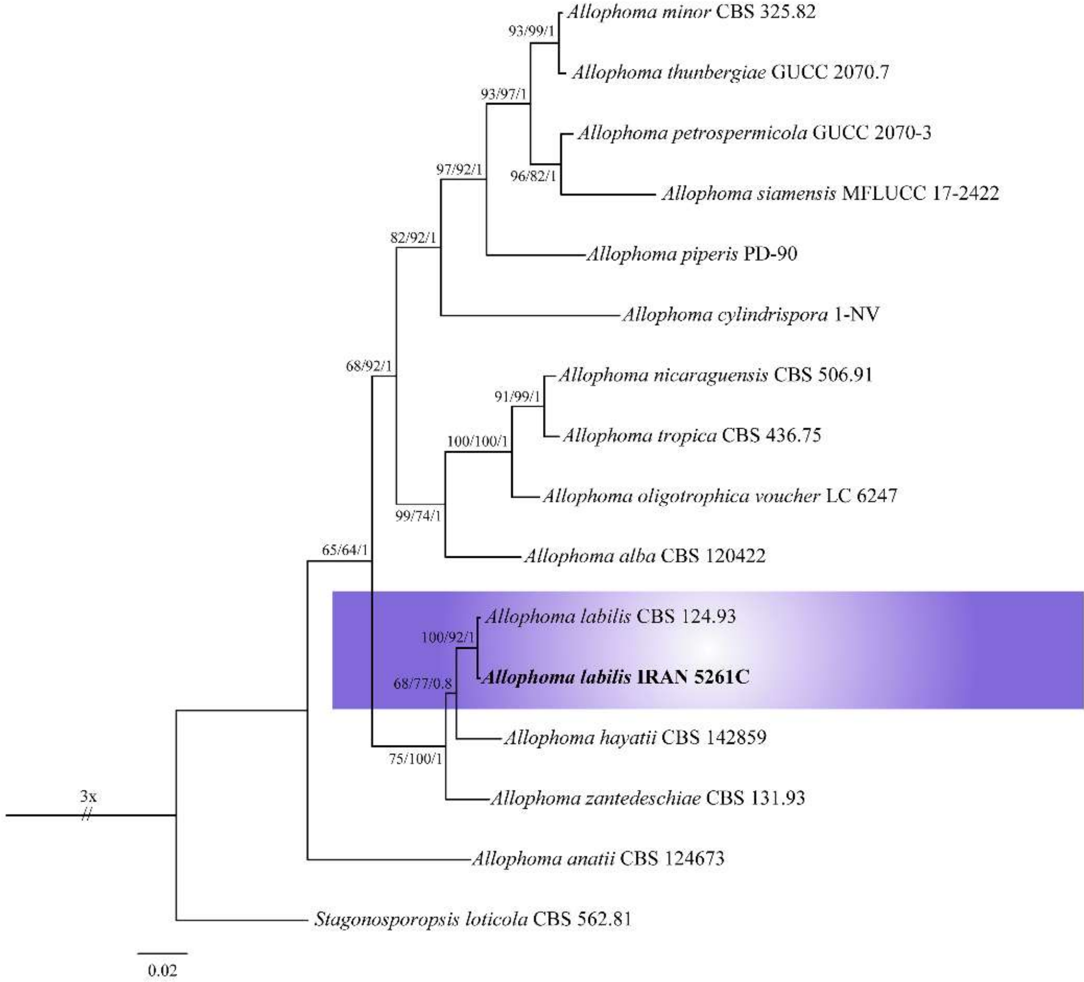

Fig. 3. One of the three equally most parsimonious phylogenetic trees inferred from the *rpb2* locus sequence data across *Allophoma* species. The tree was rooted to *Stagonosporopsis loticola* CBS 562.81. The scale bar indicates the estimated number of nucleotide substitutions per site. Bootstrap support values from Maximum Likelihood (ML) and Maximum Parsimony (MP), along with posterior probabilities from Bayesian Inference (BI), are presented at the nodes as ML-BS/MP-BS/BI-PP.

Cladosporium ramotenellum K. Schub., Zalar, Crous and U. Braun, Stud. Mycol. 58:137 (2007).

The taxonomic position of the *Cladosporium* sp. Isolate (IRAN 5263C) was determined using *tef1-α*. Following alignment, the dataset comprised 353 characters, including the alignment gaps. Among these, 168 characters were constant, 80 were variable but parsimony-uninformative, and 105 were parsimony-informative. Maximum parsimony (MP) analysis of the 105 parsimony-informative characters yielded 39 parsimonious trees (TL=531, CI=0.573, RI=0.617, HI=0.427). An IQ-TREE best tree (log-likelihood -2348.003) was obtained after iterations. The best evolutionary model selected by the Model Finder in IQ-TREE was TN+F+G4. Bayesian analyses of the *tef1-α* locus formed 20,602 trees, of which 5,150 were eliminated as burn-in trees. The consensus tree and posterior probability values (PP) were calculated from the remaining 15,452 trees. The average standard deviation of split frequencies at the end of the run was 0.008648. Based on phylogenetic analyses, isolate IRAN 5263C clustered in a clade containing *Cladosporium ramotenellum* strain CPC 12047 (Fig. 4). Among 20 *Cladosporium* isolates, 12 were identified as *C. ramotenellum*. These were obtained from asymptomatic pods of the 'Bivanij' chickpea genotype. The isolates were collected from a single geographical area in Kermanshah Province,

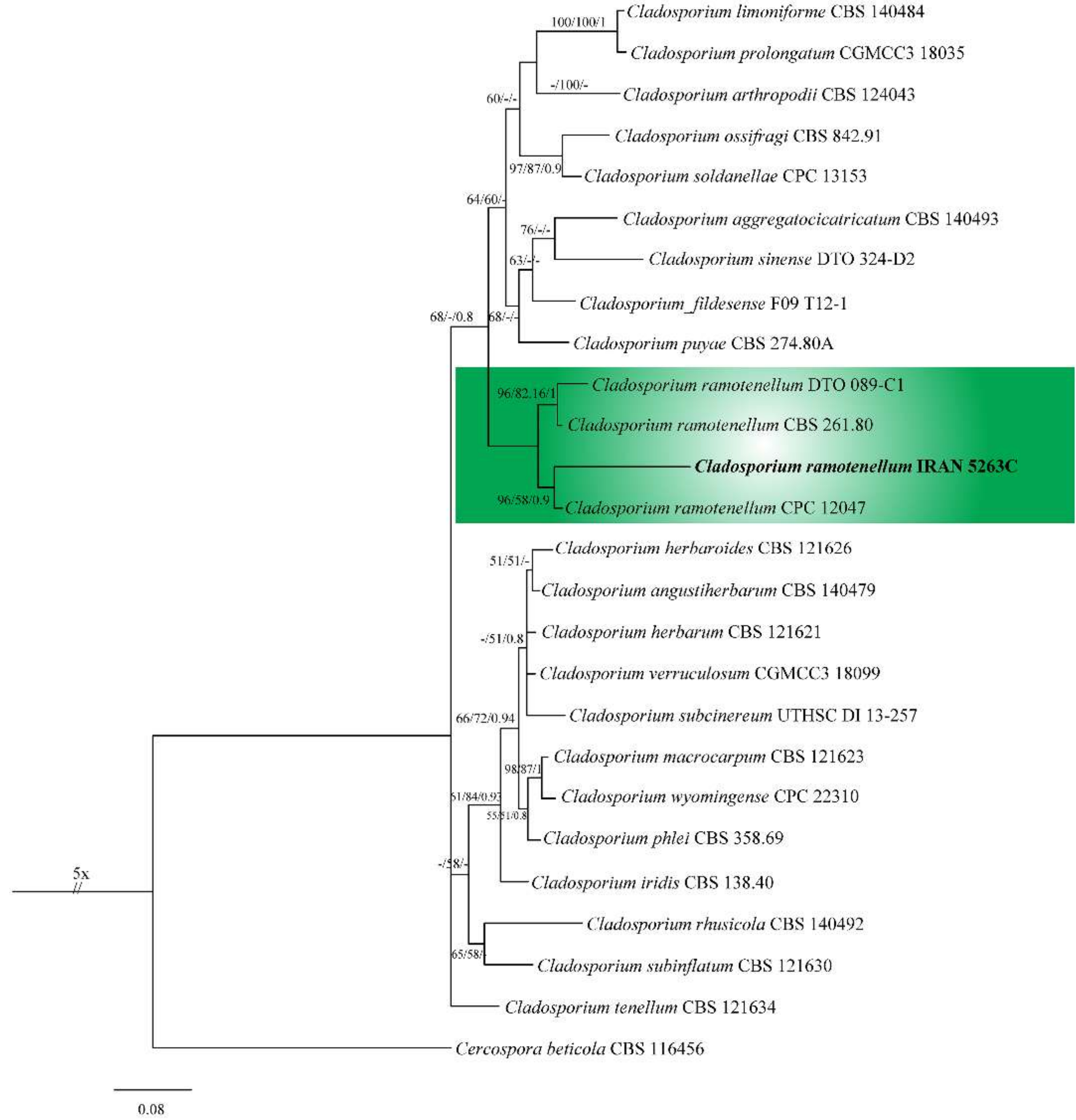

Fig. 4. One of the 39 equally parsimonious phylogenetic trees derived from the *tef1-α* genomic locus sequence data across *Cladosporium* species. The tree was rooted to *Cercospora beticola* (CBS 116456). The scale bar indicates the estimated number of nucleotide substitutions per site. Bootstrap support values from Maximum Likelihood (ML) and Maximum Parsimony (MP), along with posterior probabilities from Bayesian Inference (BI), are presented at the nodes as ML-BS/MP-BS/BI-PP.

Iran: Znylaan Sofla.

Note: Hu (2023) reported *Cladosporium ramotenellum* as an agent of Sooty spot on postharvest Clementines in the United States. Sandoval-Denis et al. (2015) identified *C. ramotenellum* as a human pathogen. This fungal species was also obtained from *Micromeria graeca* as saprobe (Zimowska et al. 2021).

Aspergillus* spp. (**including ***Aspergillus luchuensis Inui, J. Coll. Sci. Imp. Univ. Tokyo 15: 469 (1901); ***Aspergillus tubingensis*** Mosseray, Cellule 43: 245-247 (1934); ***Aspergillus welwitschiae*** (Bres.) Henn. Hobraken et al., Stud.Mycol.107: 27 (2020); ***Aspergillus fumigatus*** Fresen., Beiträge zur Mykologie 3: 81 (1863).

The taxonomic positions of the representative *Aspergillus* sp. isolates (IRAN 5264C, IRAN 5265C, IRAN 5266C, IRAN 5267C) were determined using *cal*. Following alignment, the dataset comprised 698 characters, including alignment gaps. Of these, 245 characters were constant, 119 were variable but parsimony-uninformative, and 334 were parsimony-informative. Maximum parsimony (MP) analysis of the 334 parsimony-informative characters yielded 12 parsimonious trees (TL=1016, CI=0.745, RI=0.914, HI=0.255). An IQ-TREE best tree (log-likelihood -4496.1252) was obtained after iterations. The best

evolutionary model selected by Model Finder in IQ-TREE was TIM2e+I+G4. Bayesian analyses of the *cal* locus yielded 19,402 trees, of which 4,850 were eliminated as burn-in trees. The consensus tree and posterior probability values (PP) were calculated from the remaining 14,552 trees. The average standard deviation of split frequencies at the end of the run was 0.009660. Based on phylogenetic analyses, isolates IRAN 5264C, IRAN 5265C and IRAN 5266C clustered in well-supported clades containing *Aspergillus luchuensis* strain CBS 205.80, *Aspergillus tubingensis* strain NRRL 4875 and *Aspergillus welwitschiae* strain CBS 139.54 in *Nigri* section, respectively. Moreover, isolate IRAN 5267C clustered in a well-supported clade containing *Aspergillus fumigatus* strain CBS 133.61 in *Fumigati* section (Fig. 5). A total of 260 *Aspergillus* isolates were recovered, of which 14 isolates belonged to *A. luchuensis* from stems of the 'Bivanij' genotype cultivated in two geographical area in Kermanshah Province, Iran: Znylaan Sofla, Anjirak, 50 isolates belonged to *A. tubingensis* isolated from roots, stems and leaves of 'Sufi', 'Line', 'Desi-17', 'Flip09-2780' and 'KaKa' genotypes cultivated in greenhouse with and without seed disinfection, 17 isolates belonged to *A. welwitschiae* from seeds and stems of 'Desi-17' and 'Flip09-2780' genotypes cultivated in a geographical area located in Hamedan Province, Iran: Tuyserkan and 5 isolates belonged to *A. fumigatus* from leaves of 'Flip09-2780' genotype cultivated in greenhouse with seed disinfection.

Note: Tovar- Sanchez et al. (2023) reported *Aspergillus luchuensis* as an endophyte in *Prosopis laevigata.* Nisa et al. (2020) identified *A. tubingensis* as endophyte in *Debregeasia salicifolia. A. welwitschiae* was identified as endophyte in *Ficus retusa* (Moglad et al. 2023). Moreover, Alijani et al. (2016) reported *A. welwitschiae* as an endophyte in apple in Iran. In terms of *A. fumigatus,* Liu et al. (2004) and Jiang et al. (2022) isolated this species as endophyte from *Cynodon dactylon* and *Crocus sativus L.* Furthermore, Alijani et al. (2016) obtained *A. fumigatus* as endophyte from apple trees in Iran.

Penicillium chrysogenum Thom, U.S.D.A. Bur. Animal Industr. Bull. 118: 58 (1910).

The taxonomic position of the *Penicillium* sp. isolate studied here (SMPf41) was determined using *cal* and *tub2* loci. Following alignment, the concatenated dataset comprised 943 characters, including alignment gaps. Among these, 735 were constant, 144 were variable but parsimony-uninformative, and 64 were parsimony-informative. Maximum parsimony (MP) analysis of 64 parsimony-informative characters yielded 11 parsimonious trees (TL=286, CI=0.853, RI=0.768, HI=0.147). An IQ-TREE best tree (log-likelihood -2917.541) was obtained after iterations. The best evolutionary model selected by Model Finder in IQ-TREE was TNe+G4. Bayesian analyses of the concatenated alignments of the two loci yielded 902 trees, of which 224 were eliminated as burn-in. The consensus tree and posterior probability values (PP) were calculated from the remaining 678 trees. The average standard deviation of the split frequencies at the end of the run was 0.008487. Based on phylogenetic analyses, isolate SMPf41 clustered in a well-supported clade containing *P. chrysogenum* strain DTO- 102B4 (Fig. 6). All four *Penicillium* isolates were identified as *P. chrysogenum*. They were recovered from asymptomatic stems of the 'Moghadamati-Paiizeh' chickpea genotype (*Cicer arietinum*) collected in Tuyserkan, Hamedan Province, Iran.

Note: Ansari et al. (2022) isolated *Penicillium chrysogenum* from sugarcane in Iran. Gashgari et al. (2016) reported this species as endophyte from medicinal plants in Saudi Arabia.

DISCUSSION

Given projected world population growth and rising inflation, nutrient-dense and versatile foods such as chickpeas should be incorporated into a global food pyramid to support nourishing diets for populations. However, in the context of global warming and associated environmental challenges, such as drought, crop production faces increasing difficulties, and chickpeas are not immune to these pressures. This underscores the need for resilient agricultural strategies and diversified dietary recommendations that account for climatic risk and resource availability while ensuring nutritional adequacy. Moreover, innovative approaches—such as the application of endophytic microorganisms, which exert no harmful environmental effects and enhance plant resistance to biotic and abiotic stresses—have attracted growing attention.

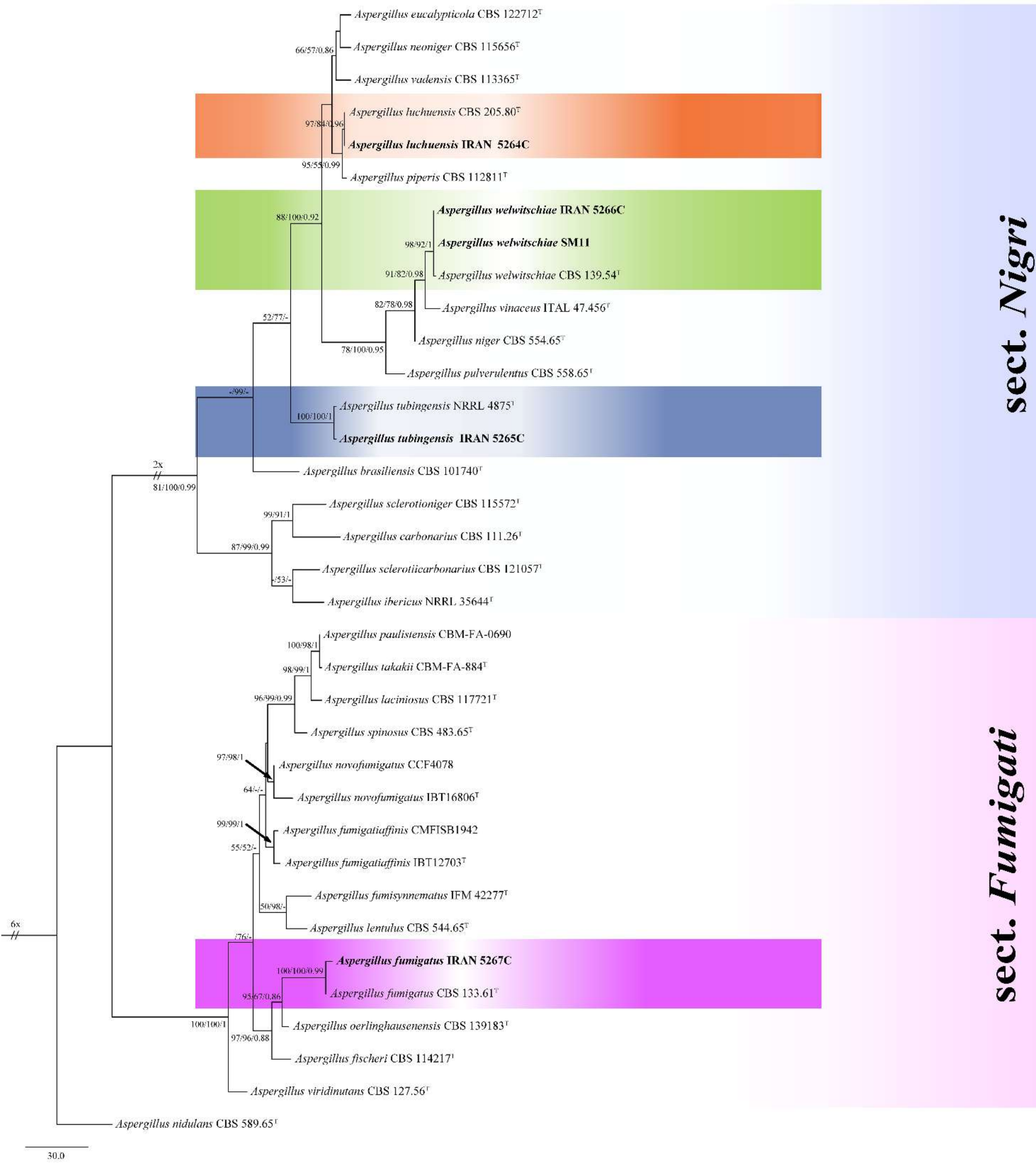

Fig. 5. One of the 12 equally most parsimonious phylogenetic trees derived from the *cal* genomic locus sequence data across *Aspergillus* species belonged to two sections (*Nigri* and *Fumigati*). The tree was rooted to *Aspergillus nidulans* CBS 589.65. The scale bar indicates the estimated number of nucleotide substitutions per site. Bootstrap support values from Maximum Likelihood (ML) and Maximum Parsimony (MP), along with posterior probabilities from Bayesian Inference (BI), are shown at the nodes as ML-BS/MP-BS/BI-PP.

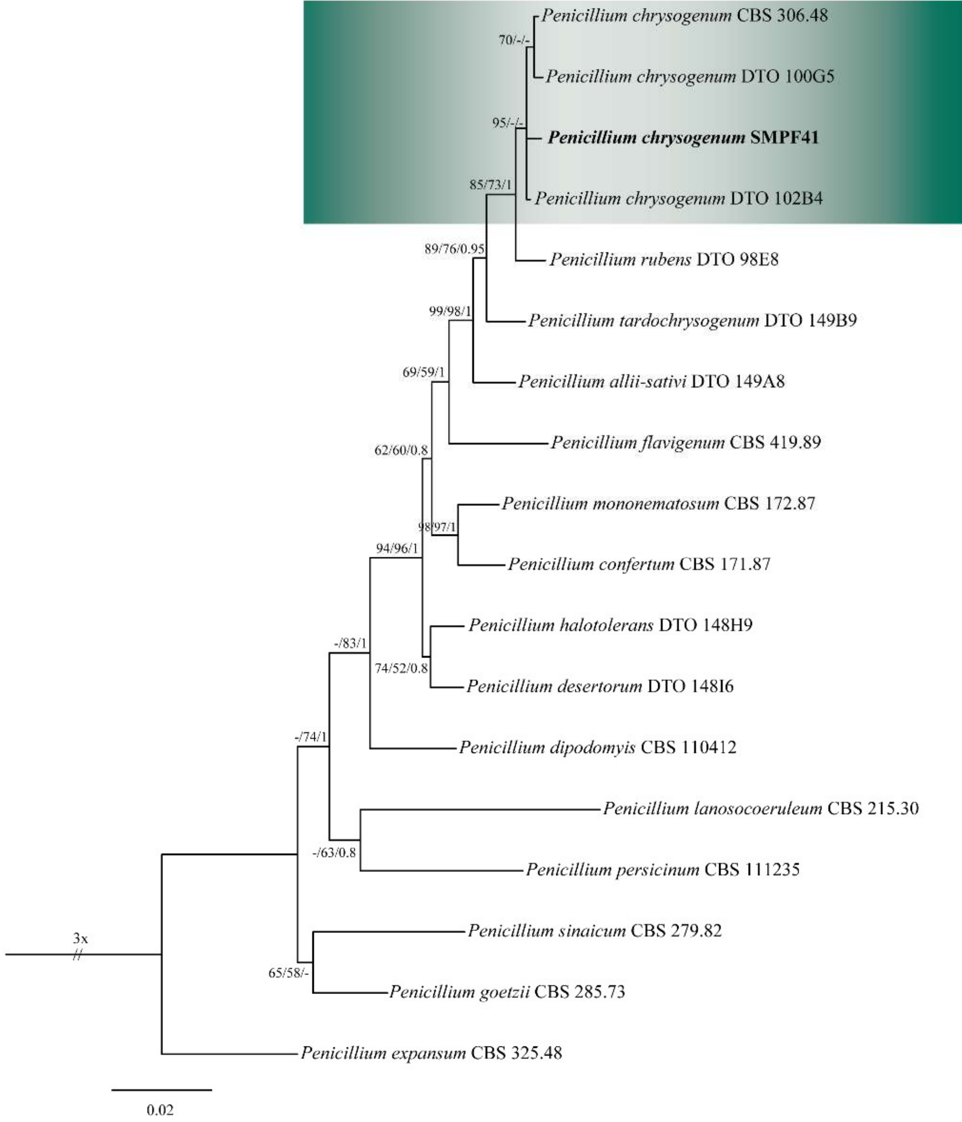

Fig. 6. One of the 11 equally most parsimonious trees derived based on combined *cal* and *tub2* loci sequence data across *Penicillium* species. The tree is rooted with *Penicillium expansum* CBS 325.48. The scale bar indicates the estimated number of nucleotide substitutions per site. Bootstrap support values from Maximum Likelihood (ML) and Maximum Parsimony (MP), along with posterior probabilities from Bayesian Inference (BI), are shown at the nodes as ML-BS/MP-BS/BI-PP.

A substantial body of work has pursued the identification of endophytic fungi across diverse crops and plants, including *Glycine max* (Impullitti and Malvick 2013, Fernandes et al. 2015), *Solanum lycopersicum* (Dong et al. 2021), and *Gynura japonica* (Riga et al. 2024).

In this study, for the first time, we identified endophytic fungi from seeds and plant organs of different genotypes of *Cicer arietinum* under natural and greenhouse conditions. Our results show that the number of isolates obtained from farm-derived samples exceeded those from greenhouse-grown plants. Among the organs from which endophytic fungi were isolated, roots from samples collected at farms in Kermanshah Providence yielded the highest portion of isolates (9.67% in irrigated and 43.87% in dryland conditions), suggesting a stronger association between roots and microorganisms due to their proximity to the rhizosphere. This finding aligns with prior reports of Wang et al. (2022) in *Sophora alopecuroides* and Ahmad et al. (2024) in *Cannabis sativa* L. Furthermore, leaves and stems from field samples collected at the field site in Hamedan Province exhibited the highest number of isolates (50%), a result that contrasts with the aforementioned studies. Similarly, the number of isolates obtained

from seeds sown without disinfection (80 isolates) under greenhouse conditions exceeded those from disinfected seeds (60 isolates) sown under the same conditions, indicating that seed disinfection reduces microbial growth. This result aligns with Davoudpour et al. (2020), who reported a significant reduction in microbial growth in *Zea mays* seeds following disinfection with ethanol and sodium hypochlorite. Moreover, among the organs from which endophytic fungi were isolated in the greenhouse-grown samples, stems from non-disinfected seeds and roots from disinfected seeds exhibited the highest numbers of isolates (47.50% and 45%, respectively).

Among the fungal genera identified in this study, *Fusarium* and *Aspergillus* were dominant in samples collected from farms in Kermanshah Providence, accounting for 60% and 27.09%, respectively. Similarly, *Aspergillus* sp. was dominant at 66% from farm samples in Hamedan Providence. Under greenhouse conditions, *Aspergillus* was the dominant genus in seeds sown with and without disinfection, representing 91.66% and 100%, respectively.

Among the various genotypes of chickpea seeds examined, a total of 21 fungal isolates were recovered, predominantly belonging to the genera *Aspergillus* (57.14%) and *Cladosporium* (42.85%). The 'Flip09-2780' and 'Desi37' genotypes yielded the highest number of isolates (seven isolates). Similarly, in field-grown genotypes from Hamden Province, genotypes 'Adel' and 'Moghadamati-Paiizeh' exhibited the highest isolate frequency (30%).

Furthermore, under greenhouse conditions, the genotype 'Kaka' exhibited the highest proportion of isolates in non-disinfected seeds (26.25%), whereas 'Flip09-2780' showed the greatest proportion of isolates in disinfected seeds (31.66%). All fungal isolates in this study were recovered from asymptomatic *Cicer arietinum* plants. However, since some of the identified species have been reported as pathogens in other contexts, future pathogenicity tests are necessary to conclusively confirm their endophytic nature in chickpea.

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AUTHOR CONTRIBUTION

Project administration: Mohammad Javan-Nikkhah; Writer: Mohammad Hojati; Review and editing: Mohammad Javan-Nikkhah; Investigation: Mohammad Hojati; Operator: Mohammad Hojati; Software: Zahra Rastaghi.

DATA AVAILABILITY

All data are available in online repositories. Requests for more data and materials should be addressed to Mohammad Javan-Nikkhah.

DECLARATION

The authors declare that there is no conflict of interest.

FUNDING

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ETHICS APPROVAL

Not applicable.

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قارچ‌های اندوفیت همراه نخود (*Cicer arietinum* L.) در ایران

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چکیده

نخود (*Cicer arietinum*) یکی از حبوبات مهم است. این گیاه یک‌ساله در بسیاری از مناطق جهان به‌طور گسترده کشت می‌شود. یکی از ویژگی‌های قابل توجه نخود، ارتباط همزیستی متنوع آن با میکروارگانیسم‌های گوناگون است که به باروری خاک کمک می‌کنند. در این مطالعه، تنوع قارچ‌های اندوفیت موجود در دانه‌ها، اندام‌های هوایی و زیرزمینی بیست و یک ژنوتیپ نخود بررسی شد. ژنوتیپ‌ها از مؤسسه تحقیقات کشاورزی مناطق خشک (مراغه، استان آذربایجان شرقی، ایران) تأمین شدند. این ژنوتیپ‌ها در شرایط گلخانه‌ای و مزرعه‌ای با استفاده از دانه‌های ضدعفونی‌شده و غیرضدعفونی‌شده کشت شدند. علاوه بر این، برای ارزیابی تنوع قارچ‌های اندوفیت در شرایط مزرعه، نمونه‌برداری در مزارع نخود در روستاهای انجیرک و زنیلان‌سفلی شهرستان کرمانشاه و همچنین در مزارع پردیس آزمایشی دانشکده کشاورزی دانشگاه رازی، کرمانشاه، ایران انجام شد. در مجموع، ۳۶۶ جدایه قارچی به‌دست آمد که از میان آنها، ۸۰ جدایه از نمونه های بدون ضدعفونی و ۶۰ جدایه از نمونه های ضدعفونی‌شده گیاهان کشت شده در گلخانه به‌دست آمد. علاوه بر این، از ژنوتیپ‌های نخود کشت‌شده در مزرعه (استان همدان) و ۴۸ نمونه گیاهی جمع‌آوری‌شده از شش مزرعه در استان کرمانشاه، به ترتیب ۵۰ و ۱۵۵ جدایه قارچی اندوفیت به‌دست آمد. همچنین، ۲۱ جدایه قارچی از بذر ژنوتیپ‌های «Desi 37»، «Kaka»، «Sufi» و «Flip 09-2780» به دست آمد. پس از بررسی ریخت شناختی و توالی‌یابی جدایه‌های نماینده، بر اساس نواحی ژنومی *tef1-α*، *tub2*، ITS rDNA، *cal* و *rpb2*، ده گونه قارچی متعلق به شش جنس شناسایی شدند. گونه‌های شناسایی‌شده عبارتند از *Allophoma labilis*، *Aspergillus fumigatus*، *A. luchuensis*، *A. niger*، *A. tubingensis*، *Chaetomium rectangulare*، *Cladosporium ramotenellum*، *Fusarium acuminatum*، *F. redolens* و *Penicillium chrysogenum*. بیشترین جدایه‌ها متعلق به جنس‌های *Aspergillus* (۶۰/۶۵ درصد) و *Fusarium* (۲۷/۸۶) بودند. در میان ژنوتیپ‌های نخود، بیشترین تعداد جدایه‌ها از ژنوتیپ‌های «Flip 09-2780» (۴۳ جدایه) و «Adel» (۴۱ جدایه) به‌دست آمد.

کلمات کلیدی: آسپریلوس، ارتباط همیاری، تنوع زیستی قارچ‌ها، حبوبات، فوزاریوم.

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