PHYSIOLOGICAL AND MOLECULAR CHARACTERIZATION OF *Ascochyta rabiei* ISOLATES FROM VARIOUS CHICKPEA AREAS ACROSS IKR, IRAQ

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ABSTRACT

From 2017 to 2019, 51 *A. rabiei* isolates were isolated from 259 chickpea fields across IKR. On different media, 32 isolates showed significant differences in morphological characteristics. The isolates were divided into six groups based on colony color, five groups on mycelium color and three groups on pycnidia color. CSMDA was the best media for mycelial growth. AS-28 significantly surpassed all other isolates in colony diameter despite media type. *A. rabiei* growth varied between 15-35°C, the maximum growth occured at 25°C and ceased at 35°C. The mean conidia and pycnedia dimensions in isolates AS-19 and AS-9 ranged from 20.0*7.5µm and 70.8*47.9µm to 21.8*9.0µm and 140.7*93.6µm in AS-11 and AS-18 respectively. The isolates were classified into four groups and 15 races based on pathogenicity and virulence. Race 1 exhibited high aggressiveness and virulence against all differentials, whereas the other races explored variable virulence spectrum. Sulaimani had the greatest *A. rabiei* diversity, with nine different races accounting for 60% of population, followed by Erbil with five races (33%). Halabja and Garmian each contribute three and two races, accounting for 20% and 12% of the total. Races 4 and 5 were the most populous and widely spread in IKR. The ITS region was amplified to a541bp band in all *A. rabiei* isolates. The length of the nucleotide sequences ranged from 481 to 541bp. The ITS sequences of all the isolates were registered at the NCBI Gen Bank under different accession numbers. The phylogenetic tree clearly shows that all the isolates are grouped in one cluster and have a high degree of similarity.

Key words: Aschochta blight disease, *Cicer arietinum*, Physiological races, molecular diagnosis, fungal disease.

التوصيف الفسيولوجي والجزيئي لعزلات الفطر *Ascochyta rabiei* من مناطق انتاج الحمص المختلفة في كردستان العراق

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المستخلص

خلال الفترة 2017 إلى 2019، تم عزل 51 عزلة من الفطر *A. rabiei* من 259 حقل حمص في إقليم كردستان. أظهرت 32 عزلة فروقات معنوية في الخصائص المظهرية على اوساط غذائية مختلفة. صنفت عزلات الفطر لست مجاميع اعتمادا على لون المستعمرة، خمس مجاميع اعتمادا على لون الخيوط الفطرية، وثلاث مجاميع وفق لون البيكنيديا. كان الوسط الغذائي CSMDA أفضل بيئة لنمو الفطر. تفوقت العزلة AS-28 معنويا على جميع العزلات في متوسط قطر المستعمرة بغض النظر عن الوسط المستخدم. تباين نمو الفطر *A. rabiei* عند مدى حراري 15-35 °م، سجل اعلى معدل نمو عند 25°م وتوقف عند 35°م. تراوح متوسط أبعاد السبورات الكونيدية والبكنيديا في العزلات AS-19 وAS-9 من 20.0*7.5 ميكرون و70.8*47.9 ميكرون إلى 21.8*9.0 ميكرون و140.7*93.6 ميكرون في العزلات AS-11 وAS-18 على التوالي. صنفت عزلات الفطر الى أربع مجاميع و15 سلالة اعتمادا على ضراوتها وفوعتها المرضية. اظهرت السلالة الأولى ضراوة وفوعة عالية على جميع الأصناف التفريقية، بينما استكشفت السلالات الأخرى عن طيف متغاير من الفوعة. تميزت السليمانية بتنوع وراثي عالي وضمت تسع سلالات مختلفة تمثل 60٪ من السكان، اعقبه أربيل بخمس سلالات (33٪). بينما ساهم كل من حلبجة و كرميان بثلاثة سلالات وسلالتين وبنسبة 20٪ و12٪ على التوالي. كانت السلالات 4 و5 أكثرها شيوعا وانتشارا في إقليم كردستان. تم تضخيم منطقة ITS عن حزمة 541bp في جميع عزلات الفطر *A. rabiei*. تراوح طول تسلسل النيوكليوتيدات من 481 إلى 541 زوج قاعدي. تم تسجيل تسلسل ITS لجميع العزلات في بنك الجينات العامNCBI تحت أرقام انضمام مختلفة. اظهر شجرة النشوء والتطور بوضوح أن جميع عزلات الفطر تجتمع في عنقود واحد وتتقاسم درجة عالية من التشابه.

كلمات مفتاحية: لفحة الاسكوكايتا، *Cicer arietinum*، سلالات فسلجية، تشخيص جزيئي، امراض فطرية.

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INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a native Asian pules plant that is grown as the most important cash and food security crop in the drylands of West Asia and Indian subcontinent (30). It is an important protein source in many parts of central Asia and Africa, and it is thought to be one of the first grain legumes domesticated in the old world, having originated in southeastern Turkey and northern Syria (1). It can be used as a common carbohydrate and protein source, making it more cost-effective and affordable for developing countries while maintaining nutritional quality (29). From 2008 to 2017, the average annual production shares of chickpeas by region revealed that Asia acounted for 83% of the global chickpea production (16). The crop is affected by various pathogens including fungi, viruses, nematodes and other pests such as insects. Several diseases, including Ascochyta blight (AB) caused by *Ascochyta rabiei*, Fusarium wilt (FW) incited by *Fusarium oxypsorum f sp. ciceris*, and Botrytis gray mold (BGM) caused by *Botrytis cinerea*, limit chickpea production (35). AB, also known as chickpea blight, gram blight, ascochytosis, anthracnose, rabia or chickpea scorch, affects all above ground parts of the host plant. The disease is one of the most damaging to chickpeas, resulting in significant yield and quality losses (17). Cool, cloudy, humid, and rainy weather (15-25C° and >150 mm of annual rainfall) during the growing season promotes disease development, resulting in yield losses of up to 100%. The infection is spread by airborne spores. Fungicide can be used to control the disease in the majority of developing countries (26). The disease remains one of the most serious chickpea diseases in many parts of the world, particularly in Western Asia, North Africa and the Northwestern region of India and Pakistan (39). The recent widespread damage to chickpea cultivars released as resistant to AB suggests the presence of different races of the pathogen. Pathogenic variation has been reported in *A. rabiei* isolates from India, Syria, Lebanon, Pakistan, Tunisia and Israel (21, 43). The first evidence of races in *A. rabiei* was discovered in 1963, when the resistant Indian chickpea cultivar C12/34, became susceptible (32). several races were held in the Indian state of Punjab according to Bedi and Aujla (9). Vir and Grewal, (44) used five differential cultivars to identify races I and 2 and one biotype of race-2 in India, and this was later confirmed using three differential cultivars (19). Qureshi and Alam (37) discovered five races of *A. rabiei* in Pakistan, whereas Reddy et al. (40**)** reported that some resistant chickpea genotypes developed in Syria were susceptible in Pakistan, suggesting differences between the existence races of *A. rabiei* in Pakistan and Syria. Despite significant variation in the aggressiveness of the isolates, as well as variation in the size of pycnidia, colony growth rate and sporulation in vitro, Gowen, et al. (18), found no evidence for the existence of races. By inoculating a set of six differential cultivars, six pathogenic groups or races were identified. All of the differentials in Syria, Lebanon and Italy were susceptible to race 6, suggesting that the three countries share a common race (36). Chongo et al. (13) used eight differentials to identify fourteen races of *A. rabiei* from forty isolates in Canada, whereas 13 differentials were used to identify only five races of the pathogen in Iraq (4). Fungal identification using traditional methods requires long time, effort, and knowledge of classical taxonomy knowledge and may result in unreliable results due to identification issues since it requires experts and specialists with experience (11). A wide range of molecular methods, based on genotypic characteristics, are increasingly becoming valuable tools in all aspects of fungal diagnostics, providing fast, highly specific, effective, and potentially more accurate results. Nucleic acid-based methods enable the identification of closely related species as well as fungal races [40]. The current study was conducted to identify the physiological specialization in *A. rabiei* isolates from IKR, Iraq, as well as to detect any genetic variation among pathogen isolates.

MATERIALS AND METHODS

Isolation and purification of *A. rabiei* isolates: Infected ABD samples collected from chickpea stem, branches, pod, and seeds were used for isolation *A. rabiei*. Samples were collected during 2017-2019 from 259 fields in 29 districts of Sulaymaniyah, Garmian,

Halabja, and Hawler provinces IKR, Iraq. Chickpea seed meal dextrose agar (CSMDA) was used for isolation and purification of the isolates (41). The infected samples were cut into 1 cm pieces, surface sterilized with 0.5% NaOCl for 2-3 min., rinsed three times in sterilized distilled water(SDW), dried with sterile filter paper for one minute and plated on CSMDA. Plates were incubated upside down at 20°C. After four days, a cork borer used to cut a 5 mm dia. agar plug from the margin of an actively growing culture and transfer it to CSMDA plates (10). Inoculated plates were incubated at 20±2°C for 2 weeks (46). Each isolate was purified as single spore culture and incubated for two weeks at 22±2°C. Pycnidial fungi were tentatively identified using Sutton key**.** *A. rabiei* single spore isolate colonies were preserved on CSMDA slants at 4°C as short term storage for further studies(22). While long term preservation was conducted by placing the pycnidiospores in a solution of $5mgL^{-1}$ skim milk powder dissolved in 25% glycerol and water and stored in cryofreezer at -80°C until used for DNA extraction.

Cultural and Morphological Characteristics

All the isolates, were morphologically examined visually or with microscope including colony morphology, conidia and pycnidia shape and size, and mycelial growth. Effect of eight culture media, CSMDA, Oat meal agar (OMA), Potato dextrose agar (PDA), Corn meal agar (CMA), Czapex-Dox+lignocellulose agar (COMPLETE), Nutrient Agar (NA), Sabouraud Dextrose Agar (SDA), and MacConkey agar (MA) were studied on growth rate, colony color and zonation patterns of different *A. rabiei* isolates, While CSMDA, OMA and PDA were only used to study the conidiospore and pycnidium shape and size after 20 days of incubation at 25°C. A 5mm diameter disc of 7 days old culture inoculum of each isolate was transferred to 90mm petri plates center. The inoculated plates were arranged in complete randomized design (CRD)with three replicates and incubated at 20±2ºC. Growth rate of each isolate was studied at 15, 20, 25, 30 and 35°C on CSMDA. Three petri plates were used for each treatment with three replicates. Mean radial mycelium growth in centimeters was measured from the center of the inoculum disc to the tip of the hypha in four directions after 20 days of inoculation.

Phenotyping of *A. rabiei* isolates

Pathogenicity of *A. rabiei* isolates was conducted in a greenhouse covered with 200µm thick polyethylene plastic film in Bazian to pathotype the isolates. Ten chickpea differentials provided by ICARDA used to characterize physiological races of the isolates according to their virulence (41). Ten seeds of each genotype were sown in plastic pots filled with 12 kg/pot of sterilized sandy loam. The seedlings were kept in a greenhouse for 14days before inoculation. Twenty-three *A. rabiei* isolates were assessed for virulence variation in a greenhouse at 20/25°C. 14 days-old culture of each isolate grown on CSMDA at 20±2°C was flooded with 10ml of sterile water and disrupted with a sterile glass rod to release the conidia. The conidial suspension was filtered through three layers of cloth cheese to remove mycelia fragments, and the spore concentration was adjusted to $1x10^6$/ml (22). The seedlings were artificially inoculated with the spore suspension until runoff then covered with transparent polyethylene bags for 48h to maintain high humidity and promote infection. Control treatment was sprayed with sterile distilled water. Plants were kept under glass house conditions at 21-25°C and mist irrigated each 1h for 30sec to keep the humidity at 70-80%. Pots were arranged in randomized complete block design (RCBD) with three replicates. Blight severity was assessed after 21 days of inoculation using 0-9 scale described by Udupa et al. (43). The isolates were classified into different pathotypes as follows. Av=Avirulent (1-40%), MV=Moderately virulent (41-50%), V=Virulent (51-60%), HV=High virulent (61-100%). While cultivars rated based on their mean severity: R=Resistant (1-15%), T=Tolerant (16-40%), MS=Moderately Susceptible (41-50%), S=Susceptible (51-75%).

Genotyping of *A. rabiei* isolates

DNA extraction

Mycelial inoculum from pure slant of *A. rabiei* isolates was used to inoculate CSMDA plates supplemented with streptomycin sulfide (250ppm). A loop of mycelium was transferred to Eppendorf PCR tubes after

10day incubation period at 25°C (1.5 ml) or by flooding the plates with sterilized normal saline (0.9% NaCl) before pipetting the suspension into a clean sterile PCR tube, spinning and discarding the supernatant before using the pellet. The genomic DNA of 35 samples was extracted according to the Addbio DNA extraction protocol from fungal mass growing in 2-YEG broth using the prime prep genomic DNA extraction kit according to NORGEN kit fungi genomic DNA isolation kit.

DNA Quantification and purification

DNA concentration and purity was determined using spectrophotometer. The extracted DNA was placed on the device lens and measured in a double beam UV-visible spectrophotometer. DNA samples with an OD 260/280 ratio of 1.8 to 2.0. The extracted nucleic acid concentrations ranged from 66 to 533 ng/l, with (1.5-2.0 ng) purity. The extracted DNA was run on 1% agarose gel to confirm its amount. 8 l of extracted DNA was mixed with 2 l of 6x loading buffer before being electrophoresed in 1% agarose gel and stained with Ethidium bromide. The bands were visualized with UV light, and photos were taken with a gel documentation system (Sygene, UK) The DNA concentration was measured in Nanograms per liter (ng/l), as described by (14).

Agarose Gel Electrophoresis

By combining 100mL of 10X TBE with 900mL of ddH2O in a suitable container, the 10X TBE buffer was diluted tenfold to the prepare 1X TBE buffer. Followed the instructions, a one percent agarose gel was prepared for electrophoresis, and DNA was loaded onto the gel (45).

Amplification of internal transcribed spacer (ITS) region (rDNA Analysis)

A. rabiei isolates were used for rDNA analysis, nuclear ribosomal, internal transcribed spacer ITS Sequencing–primer ITS1(TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC), were used to amplify approximately 540bp of ITS1, 5.8S ribosomal gene, and ITS2 region from all the isolates using 2xEasyTaq master mix.=

Phylogenic analysis

About 12µl (35-55ng/µl) of *A. rabiei* PCR products were amplified by both ITS1 and ITS4 primers, and 5µl of each primer was placed in a 0.5ml Eppendorf tube separately, sealed tightly with parafilm and sent to Macrogen Co. South-Korea for sequencing. Capillary DNA analyzer was used to purify and sequence the product (ABI3730XL; Applied Biosystems, Japan). The obtained nucleotide sequences were then deposited in the GenBank databases (https://www.ncbi.nlm.nih.gov). Clustal W2 was used to generate phylogenic trees (27**).** (https://.www.ebi.ac.uk/Tools/phylogeny/.simple-phylogeny).

RESULTS AND DISCUSSION

Cultural and morphological characteristics of *A. rabiei* isolates

Fifty-one *A. rabiei* isolates were isolated from chickpea samples collected from 141 infected fields from 29 districts in Sulaimania, Garmian, Halabja, and Hawler across IKR. Comparison of *A. rabiei* isolates based on macroscopic and microscopic criteria reveal significant variation among 32 tested isolates. Cultural characteristics single spore culture isolates of *A. rabiei* differed on various media types, including CSMDA, OMA, PDA, CMA, and Czapx. In general, the isolates' colonies were flat and submerged, with sparse mycelium. As the pathogen grows, colony color changes in different isolates. Mycelial colonies were dense, grow slowly and initially appear creamy, then take a variety of colors depending on media type. Ability of the isolates to produce chlamydospores has been attributed to colony color variation, with numerous chlamydospores the culture turns to black. Mycelium of majority isolates were pale cream, beige, buff beige, and white buff at first, then darkened and varied in intensity at colonies' margins.

Macroscopic characteristics of *A. rabiei* isolates: Table 1 shows significant differences among *A. rabiei* isolates in morphological characteristics such as colony color, mycelial color, presence of grooves, carrot-red spore mass, zonation, conidia and pycnidia size, and mycelial growth grown on CSMDA. The isolates were divided into six groups based on colony colors, which ranged from light brown (1 isolate) to black (1 isolate), with 5 brown, 1 greenish white, 21 dark brown, and three blackish brown. The majority of colony center

colors were fluffy, flossy, or downy olive green, with only one isolate being velvet brown and two being semi-velvets. Mycelium colors varied greatly among the isolates, with some being grayish white (10 isolates), others being grayish beige (6 isolates), beige (11 isolates), buff beige (3 isolates), and creamy aspect (2 isolates). Pycnidia color ranged from brown (7 isolates) to black (1 isolate), with 21 isolates exploring dark brown color and 3 isolates exploring blackish brown (Table 1). On OMA, *A. rabiei* isolates explored differences in morphological characters. The majority of isolates were greenish brown (21 isolates), greenish white (1 isolate), and greenish olive (3 isolates), with only two isolates being grayish white and five being brown. All the colonies had fluffy greenish white centers. Mycelia of all isolates were intensely colored, ranging from creamy to white buff. (Fig.1). Pycnidia color ranged from brown (3 isolates) to dark brown (29 isolates). The majority of the colonies have concentric zonation with scattered pycnedia and grooves.Significant differences in morphological characteristics were detected between *A. rabiei* isolates on PDA. The isolates were divided into three groups based on colony colors, ranged from grayish white (3 isolates) to black (26 isolates), with only three isolates showed dark brown. The majority of colony center colors ranged from downy grayish white (28 isolates) to downy grayish (2 isolates) or velvet brown (1 isolate) to velvet dark brown (1isolate) (Fig.1). Mycelia color ranged from creamy (2 isolates) to beige (12 isolates) or creamy beige (12 isolates) and creamy aspect (2 isolates). Except two isolates that show dark brown color, all pycnedia shoed black color. Zonation has been observed in all the isolates and groves as well present in most of the isolates (Fig1). On CMA isolates colonies are distinguished by dark olive green and beige color, presence of downy or fluffy in the colony center in beige to gray, absence of grooves in most isolates, appearance of colonies surface almost smooth or velvet or semi-velvet shape, and scattering of pycnidia on colony surface.

Table 1. Morphology and culture characteristics of various *Ascochyta rabiei* isolates on CSMDA at 25°C after 20 days

Iso.	Colony color	Center color	Pycnidia color	Mycelial color	Myc. Inte.	Presence of grooves	Carrot-red spore mass	Zonation
AS-1	Dark brown	Fluffy or flossy or downy olive green	Dark brown	Creamy aspect	+++	Presence from the center towards the edge Radially furrowed	Presence or appears	Concentric zones
AS-2	Light brown	Velvet brown	brown	Beige	++	Presence on the edge	Absent	Concentric zones
AS-3	Dark brown	Semi- velvet	Dark brown	Creamy aspect	+	Presence from the middle towards the edge	Absent	Concentric zones with scattered pycnidia
AS-4	Greenish brown	Fluffy or flossy or downy grayish white	brown	Beige	+++	Presence from the center towards the edge Radially furrowed	Absent	Concentric zones
AS-5	Dark brown	Fluffy or flossy or downy grayish white	Dark brown	Beige	+++	Presence from the center towards the edge less dense	Absent	Concentric zones with scattered pycnidia
AS-6	Brown	Fluffy or flossy or downy grayish white	brown	Beige	+++	Presence from the middle towards the edge	Absent	Concentric zones with scattered pycnidia
AS-7	Brown	Fluffy or flossy or downy grayish white	brown	Grayish beige	+++	Presence on the edge	Absent	Concentric zones
AS-8	Dark brown	Fluffy or flossy or downy grayish white	Dark brown	Beige	+++	Without grooves the surface of the colony is smooth	Absent	Concentric zones with scattered pycnidia
AS-9	Blackish brown	Fluffy or flossy or downy grayish white	Blackish brown	Grayish white	+++	Presence from the center towards the edge Radially furrowed	Absent	Concentric zones
AS-10	Blackish brown	Fluffy or flossy or downy grayish white	Blackish brown	Grayish beige	+++	Presence from the center towards the edge Radially furrowed	Presence or appears	Concentric zones with scattered pycnidia
AS-11	Blackish brown	Fluffy or flossy or downy grayish white	Blackish brown	Beige	+++	Presence from the middle towards the edge	Presence or appears	Concentric zones with scattered pycnidia
AS-12	Brown	Fluffy or flossy or downy grayish white	brown	Buff beige	+++	Without grooves the surface of the colony is smooth	Presence or appears	Concentric zones with scattered pycnidia
AS-13	Dark	Semi- velvet	Dark	Grayish	+++	Presence from the middle	Absent	Concentric zones

	brown		brown	white		towards the edge less dense		with scattered pycnidia
AS-14	Brown	Fluffy or flossy or downy grayish white	Brown	Grayish white	+++	Presence from the middle towards the edge less dense	Absent	Concentric zones with scattered pycnidia
AS-15	Dark brown	Fluffy or flossy or downy grayish white	Dark brown	Buff beige	++++	Presence from the center towards the edge Radially furrowed	Presence or appears	Concentric zones
AS-16	Dark brown	Fluffy or flossy or downy grayish white	Dark brown	Grayish white	+++	Without grooves the surface of the colony is smooth	Absent	Concentric zones with scattered pycnidia
AS-17	Dark brown	Fluffy or flossy or downy grayish white	Dark brown	Grayish white	+++	Presence from the middle towards the edge	Absent	Concentric zones with scattered pycnidia
AS-18	Black	Fluffy or flossy or downy grayish white	Black	Grayish white	+++	Presence from the center towards the edge Radially furrowed	Absent	Concentric zones with scattered pycnidia
AS-19	Brown	Fluffy or flossy or downy grayish white	Brown	Grayish white	+++	Presence from the center towards the edge Radially furrowed	Presence or appears	Concentric zones with scattered pycnidia
AS-20	Dark brown	Fluffy or flossy or downy grayish white	Dark brown	Buff beige	+++	Presence from the center towards the edge Radially furrowed	Presence or appears	Concentric zones with scattered pycnidia
AS-21	Dark brown	Fluffy or flossy or downy grayish white	Dark brown	Grayish beige	+++	Without grooves the surface of the colony is smooth	Absent	Concentric zones
AS-22	Dark brown	Fluffy or flossy or downy grayish white	Dark brown	Grayish beige	+++	Without grooves the surface of the colony is smooth	Absent	Concentric zones
AS-23	Dark brown	Fluffy or flossy or downy grayish white	Dark brown	Grayish white	+++	Without grooves the surface of the colony is smooth	Absent	Concentric zones
AS-24	Dark brown	Fluffy or flossy or downy grayish white	Dark brown	Beige	+++	Without grooves the surface of the colony is smooth	Absent	Concentric zones
AS-25	Dark brown	Fluffy or flossy or downy grayish white	Dark brown	Beige	+++	Without grooves the surface of the colony is smooth	Absent	Concentric zones
AS-26	Dark brown	Fluffy or flossy or downy grayish white	Dark brown	Grayish white	+++	Presence from the center towards the edge Radially furrowed	Absent	Concentric zones with scattered pycnidia
AS-27	Dark brown	Fluffy or flossy or downy grayish white	Dark brown	Beige	+++	Without grooves the surface of the colony is smooth	Absent	Concentric zones with scattered pycnidia
AS-28	Dark brown	Fluffy or flossy or downy grayish white	Dark brown	Beige	+++	Without grooves the surface of the colony is smooth	Absent	Concentric zones with scattered pycnidia
AS-29	Dark brown	Fluffy or flossy or downy grayish white	Dark brown	Beige	++++	Without grooves the surface of the colony is smooth	Absent	Concentric zones with scattered pycnidia
AS-30	Dark brown	Fluffy or flossy or downy grayish white	Dark brown	Grayish white	+++	Presence from the middle towards the edge	Absent	Concentric zones
AS-31	Dark brown	Fluffy or flossy or downy grayish white	Dark brown	Grayish beige	+++	Presence from the middle towards the edge less dense	Absent	Concentric zones with scattered pycnidia
AS-32	Dark brown	Fluffy or flossy or downy grayish white	Dark brown	Grayish beige	+++	Presence from the middle towards the edge less dense	Absent	Concentric zones with scattered pycnidia

Colonies grown on Czapx were distinguished by dark color in general, while some isolates emerged in complete beige. Presence of grooves strongly was the most distinctive feature of the developing isolates which makes the colony surface wrinkled. The presence of pycnidia scattered on the colony surface especially at the edge, as well as the absence of carrot-red spore mass and the solid or compact colonies consistency, indicates zonation in most of the colonies (Fig 1). Cultural characteristics could not be linked to pathogenic variability. In this regard, molecular techniques may be useful in further confirming the association and correlation.

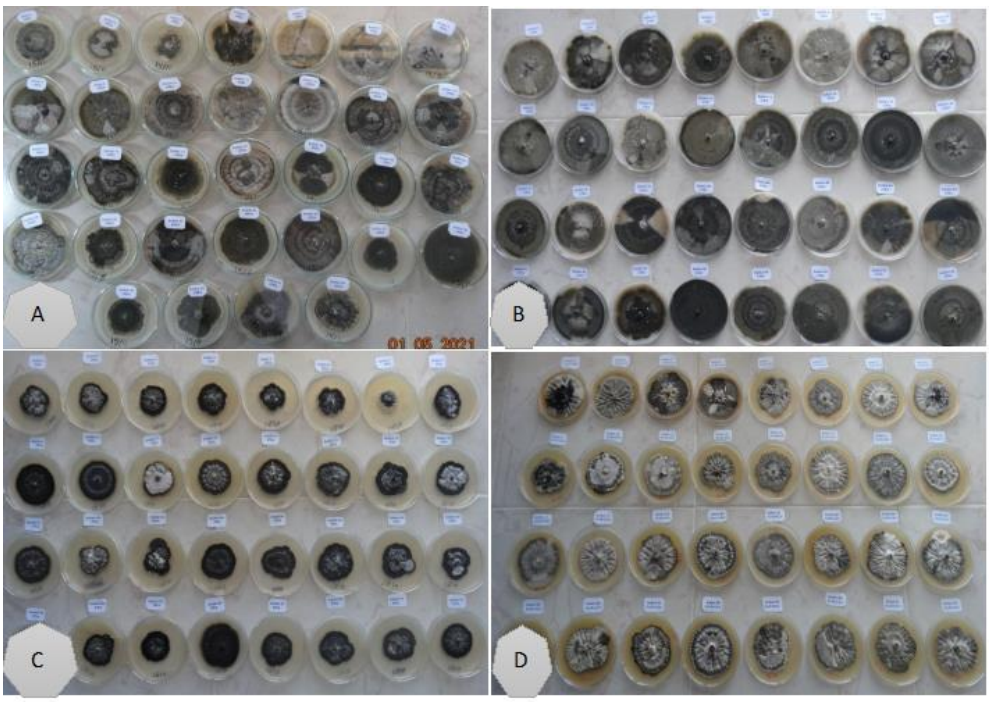

Fig.1. Colony morphology of different *A. rabiei* isolates grown on a. OMA, b. PDA, c. CMA and d. Czapx DA at 25°C after 20d

Effect of culture media on mycelial growth of *A. rabiei* isolates

Table 2 results shows that all the tested culture media significantly supports the mycelial growth of *A. rabiei* isolates to varying degrees at P≤ 0.05 after 20 days of incubation at 25°C. the mean colony diameter on the media ranged from 2.00cm on MAC to 8.04cm on OMA. Mycelial growth mean rate of the isolates on CSMDA (7.35 cm) significantly outperformed all other media's, followed by OMA (7.24cm) and Czapx (6.49cm), while the lowest growth rate was observed on MAC (2.67 cm) and NA(3.33cm). Variation in colony diameter was also observed among *A. rabiei* isolates on the used media. Isolate AS-28 significantly surpassed all other isolates in mean colony diameter (5.93cm) at P≤ 0.05 despite the used media followed by isolate AS-11. While the lowest colony diameter mean was detected in isolate AS-27 (5.16cm) followed by isolate AS-5 (5.22cm) without any significant differences (Table 2). High significant differences in growth rate among *A. rabiei* isolates were detected on CSMDA. Isolate AS-6 (7.99cm) significantly surpassed all other isolates at P≤ 0.05, except AS-11. While the lowest growth was detected in AS-19 (6.73cm). Colony diameter ranged from 6.20-8.04cm on OMA. Isolate AS-12 significantly surpassed all other isolates except AS-28. CSMDA is clearly better suited media for vegetative growth of *A. rabiei* than others, that is why widely used for multiplication and maintenance of the isolates during the study. Czapx and CMA also produced moderate mycelial growth, whereas OMA produced comparable maximum growth. The presence of chickpea seed as one of main ingredients in CSMDA which provides additional nutrients requirement for pathogen growth and development, may explain the high mycelial growth rate of *A. rabiei*. Selecting is the suitable culture media for pathogen growth, sporulation, and cultural characteristics is crucial. culture media showed significant impact on *A. rabiei* growth, pycnidia and conidia formation, and sporulation during this study. Several studies reported similar results in various fungi (31). CSMDA and OMA were found to be the best media for *A. rabiei* mycelial growth. while, PDA, moderately supported mycelial growth of the fungal isolates. This could be explained by differences in the concentrations of C, N, and other nutrients in each medium. Fresh media preparations from chickpea, oat meal, corn meal, and potato contained high concentrations of C and N sources, which promote the pathogen's maximum mycelial growth. Previous studies with various fungi have also refer to the effect of high C and N

concentrations in inducing vegetative and abundant mycelia growth (31).

Microscopic characteristics of *A. rabiei* isolates

Effect of culture media on conidia and pycnedia of the isolates

Conidia of *A. rabiei* isolates in general was hyaline, oval to oblong, and straight to slightly bent at one or both ends. Under compound microscope, they were single-celled, occasionally two-celled, and rounded at both ends. They grew on short conidiophores embedded in mucilage in a cream-pink to light-tan mass. The conidia are released from a wet mount of mature pycnidium. At moist conditions, pycnidia materials absorbs water and swollen, causing conidia to ooze out the ostiole in a slimy mass. The pycnedia is pear-shaped with a single opening called ostiole, it contains numerous hyaline unicellular and occasionally bicellular spores, Conidia and pycnedia morphology match the original described *A. rabiei* stock culture. High significant variations in conidia and pycnedia dimensions were observed among *A. rabiei* isolates on different media. CSMDA, OMA, and PDA affected conidia and pycnedia dimension differently. The highest effect was recorded in PDA, the mean conidia and pycnedia dimension (43.9*18.9μm) and (284.3*196.6μm) significantly outperformed all other media, followed by OMA (12.7*5.0μm) and (82.0*54.4μm) respectively (Table 3). Conidia and pycnedia size were high on PDA, the mean sizes of isolates (670.91 μm and 20130.9 μm) being1394% and 680% larger than those on OMA (48.4 μm and 2959.8 μm) respectively and 1600% and 711% larger than those on CSMDA (41.93μm and 2831.2μm) respectively.

Table 2. Growth rate of different *A. rabiei* isolates collected from various chickpea fields in IKR on various media type at 25°C after 20 days.

Isolate	Colony Diameter (cm)								
	CSMDA	OMA	PDA	CMA	Czapx	N.A	S.A	Mac.	Mean
AS-1	$7.7^{a.g}$	$7.02^{l.af}$	$6.35^{ah.ax}$	$6.23^{ak.bc}$	$6.59^{z.as}$	$3.22^{co.cu}$	$4.42^{cb.ck}$	$3.43^{cm.cr}$	$5.62^{b.d}$
AS-2	$7.35^{d.q}$	$6.37^{ah.ax}$	$4.97^{bt.cc}$	$6.49^{ae.au}$	$7.03^{k.ae}$	$3.37^{cm.cs}$	$5.67^{bc.bp}$	$3.34^{cm.ct}$	$5.57^{b.f}$
AS-3	$7.43^{b.n}$	$6.97^{m.ag}$	$5.3^{bk.bw}$	$6.34^{ah.ax}$	$7.02^{l.af}$	$3.83^{ck.cn}$	$4.92^{bt.ce}$	$2.11^{dd.de}$	$5.49^{c.I}$
AS-4	$7.35^{d.q}$	$7.63^{a.j}$	$5.25^{bl.by}$	$6.09^{aq.bh}$	$6.70^{u.an}$	$3.38^{cm.cs}$	$4.28^{cg.ck}$	$2.33^{cz.de}$	$5.38^{e.l}$
AS-5	$7.12^{g.ab}$	$7.12^{g.ab}$	$5.28^{bl.bx}$	$5.97^{at.bi}$	$6.71^{s.an}$	$2.92^{cq.cy}$	$4.68^{by.ch}$	2.00^{de}	$5.22^{l.m}$
AS-6	$7.99^{a.b}$	$7.64^{a.i}$	$5.07^{bq.ca}$	$6.17^{am.be}$	$6.44^{af.av}$	$2.90^{cq.cz}$	$5.59^{bf.br}$	2.02^{de}	$5.48^{c.j}$
AS-7	$7.27^{f.v}$	$6.68^{v.ap}$	$5.70^{ba.bo}$	6.26^{ai-bb}	$5.42^{bi.bt}$	$3.03^{cq.cy}$	$5.37^{bk.bu}$	$3.03^{cq.cy}$	$5.34^{g.m}$
AS-8	$7.62^{a.k}$	$7.61^{a.k}$	$4.53^{ca.ci}$	$6.69^{u.ao}$	$6.52^{ac.au}$	$3.18^{co.cw}$	$5.01^{br.ca}$	$2.92^{cq.cy}$	$5.51^{c.h}$
AS-9	$7.05^{j.ae}$	7.19^{f-x}	$5.29^{bl.bw}$	$6.66^{w.aq}$	$6.13^{an.bh}$	$3.2^{co.cv}$	$4.83^{bu.cg}$	$2.28^{db.de}$	$5.31^{h.m}$
AS-10	$7.08^{h.ad}$	$7.23^{f.x}$	$5.58^{bh.bs}$	$6.52^{ac.au}$	$6.18^{am.be}$	$3.02^{cq.cy}$	$4.56^{ca.ci}$	$2.77^{ct.dc}$	$5.37^{f.m}$
AS-11	$7.87^{a.e}$	$7.30^{e.r}$	$5.27^{bl.bx}$	$6.34^{ah.ax}$	$6.64^{y.as}$	$3.65^{cl.cp}$	$6.17^{am.bg}$	$2.88^{cq.da}$	5.76^{Ab}
AS-12	$7.38^{c.p}$	8.04^{a}	$5.36^{bk.bu}$	$6.66^{w.aq}$	$6.50^{ad.au}$	$3.43^{cm.cq}$	$4.66^{bz.ch}$	$2.46^{cy.cd}$	$5.56^{b.f}$
AS-13	$7.42^{b.o}$	$7.23^{f.x}$	$5.33^{bk.bv}$	$6.56^{aa.as}$	$6.11^{ap.bh}$	$3.92^{cj.cm}$	$5.58^{bg.bs}$	$2.61^{cw.dd}$	$5.60^{b.d}$
AS-14	$7.20^{f.y}$	$7.16^{g.z}$	$5.15^{bo.bz}$	$5.32^{bk.bv}$	$6.27^{ai.bb}$	$3.37^{cm.cs}$	$4.77^{bv.ch}$	$2.87^{cq.da}$	$5.26^{k.m}$
AS-15	$7.33^{d.q}$	$7.10^{h.ac}$	$5.35^{bk.bv}$	$5.88^{av.bk}$	$6.37^{ah.ax}$	$2.82^{cs.db}$	$4.51^{ca.ci}$	$2.84^{cr.db}$	$5.27^{j.m}$
AS-16	$7.47^{a.n}$	$7.27^{f.u}$	$4.82^{bu.cg}$	$6.56^{aa.as}$	$6.37^{ah.ax}$	$3.02^{cq.cy}$	$4.92^{bt.ce}$	$3.40^{cm.cs}$	$5.48^{c.j}$
AS-17	$7.07^{i.ae}$	$6.5^{ad.au}$	$5.62^{be.bq}$	$6.59^{z.as}$	$6.71^{s.an}$	$3.17^{co.cw}$	$6.70^{t.an}$	$2.30^{da.de}$	$5.58^{b.e}$
AS-18	$7.62^{a.j}$	$7.48^{a.n}$	$6.06^{as.bh}$	$6.24^{ak.bc}$	$6.41^{ag.av}$	$4.22^{ch.cl}$	$4.78^{bv.ch}$	$2.48^{cy.cd}$	$5.66^{b.c}$
AS-19	$6.73^{r.am}$	$7.26^{f.v}$	$4.95^{bt.cc}$	$6.80^{p.ak}$	$6.79^{q.al}$	$3.68^{cl.co}$	$4.7^{bx.ch}$	$3.37^{cm.cs}$	$5.45^{c.g}$
AS-20	$7.43^{b.n}$	$7.24^{f.w}$	$5.47^{bi.bt}$	$6.32^{ai.ay}$	$6.68^{v.ap}$	$4.35^{cd.ck}$	$4.8^{bu.ch}$	$2.97^{cq.cy}$	$5.66^{b.c}$
AS-21	$7.52^{a.m}$	$6.98^{l.ag}$	$4.92^{bt.ce}$	$6.07^{ar.bh}$	$6.84^{o.al}$	$2.82^{cs.db}$	$5.22^{bm.bz}$	2.00^{de}	$5.29^{i.m}$
AS-22	$7.67^{a.h}$	$7.67^{a.h}$	$5.38^{bj.bu}$	$5.96^{au.bj}$	$6.38^{ah.aw}$	$3.37^{cm.cs}$	$4.53^{ca.ci}$	$2.62^{cv.dd}$	$5.45^{d.k}$
AS-23	$7.02^{l.af}$	$6.20^{am.be}$	$5.79^{ax.bm}$	$6.17^{am.bf}$	$6.51^{ad.au}$	$3.15^{co.cw}$	$5.20^{bn.bz}$	$2.50^{cx.de}$	$5.32^{h.m}$
AS-24	$6.83^{o.aj}$	$7.94^{a.c}$	$4.33^{ce.ck}$	$6.7^{t.an}$	$6.66^{w.aq}$	$3.32^{cn.ct}$	$5.72^{az.bo}$	$3.23^{co.ct}$	$5.59^{b.d}$
AS-25	$7.50^{a.n}$	$7.18^{f.y}$	$5.25^{bl.by}$	$7.17^{f.z}$	$6.30^{ai.az}$	$3.00^{cq.cy}$	$4.66^{bz.ch}$	$2.77^{ct.dc}$	$5.48^{c.j}$
AS-26	$7.29^{e.s}$	$7.75^{a.f}$	$4.72^{bw.ch}$	$6.50^{ad.au}$	$6.23^{ak.bc}$	$3.30^{cn.ct}$	$4.40^{cc.ck}$	$2.51^{cx.de}$	$5.34^{g.m}$
AS-27	$7.43^{b.n}$	$7.20^{f.y}$	$4.50^{ca.cj}$	$6.25^{aj.bc}$	$6.21^{al.bd}$	$3.28^{cn.ct}$	$4.32^{cf.ck}$	$2.11^{dd.de}$	5.16^{m}
AS-28	$7.36^{c.q}$	$7.89^{a.d}$	$5.82^{aw.bl}$	$6.72^{r.am}$	$7.15^{g.aa}$	$3.25^{cn.ct}$	$6.10^{aq.bh}$	$3.13^{co.cw}$	5.93^{a}
AS-29	$6.92^{n.ah}$	$7.30^{e.r}$	$4.90^{bt.cf}$	$6.55^{ab.at}$	$6.26^{ai.bb}$	$3.10^{co.cw}$	$5.44^{bi.bt}$	$2.50^{cx.de}$	$5.37^{f.l}$
AS-30	$7.35^{d.q}$	$6.58^{z.as}$	$4.93^{bt.cd}$	$6.70^{t.an}$	$6.65^{x.ar}$	$3.27^{cn.ct}$	$5.75^{ay.bn}$	$2.63^{cu.dd}$	$5.48^{c.I}$
AS-31	$7.25^{f.w}$	$7.57^{a.l}$	$5.00^{bs.cb}$	$6.27^{ai.ba}$	$6.59^{z.as}$	$4.05^{ci.cl}$	$5.68^{bb.b}$	$2.87^{cq.d}$	$5.66^{b.c}$
AS-32	$7.63^{a.j}$	$7.28^{e.}$	$5.08^{bp.ca}$	$6.11^{ao.bh}$	$6.37^{ah.ax}$	$3.08^{cp.cx}$	$5.63^{bd.b}$	$2.2^{dc.de}$	$5.42^{d.l}$
Mean	7.35^{a}	7.24^{b}	5.23^{e}	6.37^{d}	6.49^{c}	3.33^{g}	5.11^{f}	2.67^{h}	5.47

*** Each no. is a mean of three replicates. ** Numbers followed by the same symbols significantly are not different at 0.05 level.**

Despite the media, isolate AS-11 had the largest conidial dimension (21.8*9.0μm) and size (305.50μm) and significantly outperformed all other isolates except isolate AS-12 with dimension and size of 21.7*8.9 μm and 296.61 μm, and isolate AS-9, with 20.1*9.5 μm and 290.59 μm respectively. While isolate AS-19 (202.29μm) and isolate

AS-5 (19.0*7.6*μm*) had the smallest conidial size. On the other hand, the largest means of pycnedia dimension (140.7*93.6*μm*) and size (20565.1*μm*) was recorded on isolate AS-18 and significantly outperformed all other isolates, followed by isolate AS-2 with dimension (123.9*86.9*μm*) and size (14581.5*μm*), and isolate AS-9 with the lowest dimension and size (70.81*47.9*μm*) and 4143.5 *μm* respectively. Conidial dimensions on CSMDA ranged from 9.5-12.1*3.5-4.4*μm*. The maximum conidia size recorded in AS-20 (53.6*μm*) which significantly outperformed all other isolates, and the smallest size was found in AS-5 (33.92*μm*). Pycnidia dimensions ranged from 43.9-86.5* 27.4-58.5*μm* on CSMDA. The highest pycnidia size was recoded in isolate AS-4 (5061.9*μm*), which significantly surpassed all other isolates, while the lowest size was found in isolate AS-9 (1206.0*μm*). Conidia dimensions ranged from 8.6-12.7*3.7-5.2*μm* on OMA. AS-20 recorded the highest conidia size (63.48*μm*) while AS-26 showed the lowest size (39.17*μm*). while pycnidia dimension ranged from 47.9-82.0*33.2-55.7µm. the maximum pycnidia size was detected in isolate AS-12 (4464*μm*) and the minimum size in isolate AS-4 (1607.7µm). Conidia and pycnedia dimensions varied greatly on PDA compared to other media's. Conidia size ranged from 36.3-43.9*13.8-19.7*μm*. The largest size (828.31*μm*) recorded in isolate AS-11, while the smallest size was found in AS-19 (506.73 *μm*). Pycnidia dimensions ranged from 114.1-284.3*81.9-196.6*μm*, the largest size observed in AS-18 (55905.1*μm*) and the lowest size in AS-9 (9356.1*μm*). It is not possible to distinguish *A. rabiei* races based on colony color and morphological traits. However, morphological variation can provide preliminary variation among the isolates, because these variations did not

Table 3. Conidia and Pycnedia dimensions of different *A. rabiei* isolates collected from various chickpea fields in IKR on various media at 25°C.

Isolate	CSMDA		OMA		PDA	
	Conidia (*μm*)	Pycnedia (*μm*)	Conidia (*μm*)	Pycnedia (*μm*)	Conidia (*μm*)	Pycnedia (*μm*)
AS-1	$10.2^{w\text{-}ae}$*$3.7^{z\text{-}ac}$	$78.3^{o\text{-}t}$*$52.1^{n\text{-}r}$	$10.9^{o\text{-}ad}$*$3.7^{z\text{-}ac}$	$70.3^{o\text{-}z}$*$50.6^{n\text{-}t}$	$37.5^{k\text{-}m}$*$16.0^{l\text{-}n}$	$182.4^{d\text{-}f}$*$135.5^{c\text{-}e}$
AS-2	$9.7^{ad\text{-}af}$*$3.8^{z\text{-}ac}$	82.3^{op}*$43.9^{o\text{-}z}$	11.4^{n}-x*$4.0^{u\text{-}ac}$	$71.7^{o\text{-}z}$*$48.8^{n\text{-}v}$	$38.5^{g\text{-}k}$*15.8^{mn}	217.9^{bc}*168.0^{b}
AS-3	10.3v-^{ae}*$3.9^{v\text{-}ac}$	$69.0^{o\text{-}z}$*$37.6^{t\text{-}ab}$	10.8o-ad*$3.9^{v\text{-}ac}$	$76.2^{o\text{-}v}$*$45.8^{n\text{-}z}$	$39.8^{c\text{-}g}$*$16.9^{i\text{-}k}$	$198.9^{c\text{-}e}$*144.9^{c}
AS-4	$10.9^{o\text{-}ad}$*$4.1^{t\text{-}ac}$	86.5^{o}*58.5^{n}	11.3o-y*$4.0^{u\text{-}ac}$	$48.5^{aa\text{-}ad}$*$33.2^{y\text{-}ab}$	$39.0^{e\text{-}j}$*$16.9^{h\text{-}k}$	130.1^{mn}*99.1^{kl}
AS-5	$9.7^{ad\text{-}af}$*3.5^{ac}	$67.5^{o\text{-}aa}$*$44.9^{n\text{-}z}$	$10.7^{p\text{-}ae}$*$3.9^{u\text{-}ac}$	$61.6^{r\text{-}ab}$*$35.8^{u\text{-}ab}$	36.5^{lm}*15.3^{no}	$154.3^{h\text{-}j}$*$127.8^{d\text{-}g}$
AS-6	$10.5^{r\text{-}ae}$*$4.3^{r\text{-}ab}$	$56.9^{w\text{-}ad}$*$37.7^{t\text{-}ab}$	$10.6^{q\text{-}ae}$*$4.0^{u\text{-}ac}$	$60.4^{t\text{-}ad}$*$38.8^{r\text{-}ab}$	$39.3^{e\text{-}i}$*$17.7^{c\text{-}i}$	201.0^{cd}*140.2^{cd}
AS-7	$11.2^{o\text{-}aa}$*$4.3^{r\text{-}ab}$	$65.4^{p\text{-}ab}$*$42.0^{o\text{-}aa}$	$11.1^{o\text{-}ac}$*$4.4^{r\text{-}aa}$	$64.6^{p\text{-}ab}$*$40.4^{q\text{-}ab}$	$39.6^{d\text{-}g}$*$17.9^{c\text{-}f}$	227.1^{b}*165.2^{b}
AS-8	$10.0^{z\text{-}ae}$*$3.95^{u\text{-}ac}$	$73.6^{oc\text{-}x*}$*$53.1^{n\text{-}q}$	$10.9^{o\text{-}ad}$*$4.4^{r\text{-}z}$	$66.5^{p\text{-}ab}$*$38.3^{r\text{-}ab}$	$38.2^{h\text{-}k}$*$15.6^{m\text{-}o}$	$173.1^{f\text{-}h}$*$124.9^{e\text{-}h}$
AS-9	$9.5^{ae\text{-}af}$*$4.1^{t\text{-}ac}$	$43.9^{ac\text{-}ad}$*27.4^{ab}	$11.2^{o\text{-}ab}$*$4.6^{q\text{-}y}$	$54.3^{y\text{-}ad}$*$34.4^{w\text{-}ab}$	$39.6^{d\text{-}g*}19.7^{a}$	114.1^{n}*81.9^{m}
AS-10	$9.8^{ac\text{-}af}$*$3.5^{ab\text{-}ac}$	$71.8^{o\text{-}z}$*$47.2^{n\text{-}y}$	$11.3^{o\text{-}z}$*$4.1^{t\text{-}ac}$	$76.2^{o\text{-}v}$*$43.0^{o\text{-}aa}$	36.3^{m}*$17.4^{e\text{-}j}$	$140.5^{j\text{-}m}$*93.0^{lm}
AS-11	$10.8^{o\text{-}ae}$*$3.78^{z\text{-}ac}$	$69.4^{o\text{-}z}$*$40.5^{p\text{-}ab}$	$10.7^{p\text{-}ae}$*$4.4^{r\text{-}aa}$	$47.9^{ab\text{-}ad}$*$34.0^{x\text{-}ab}$	43.9^{a}*18.9^{b}	$162.5^{g\text{-}i}$*$124.7^{e\text{-}h}$
AS-12	$10.4^{t\text{-}ae}$*$3.8^{y\text{-}ac}$	$63.8^{p\text{-}ab}$*$33.6^{x\text{-}ab}$	$11.2^{o\text{-}aa}$*$4.6^{q\text{-}w}$	82.0^{op}*$54.4^{n\text{-}p}$	43.6^{a} *$18.3^{b\text{-}d}$	$147.8^{i\text{-}m}$*$114.9^{g\text{-}j}$
AS-13	$10.7^{p\text{-}ae}$*$4.4^{q\text{-}z}$	$72.1^{o\text{-}y}$*$40.1^{q\text{-}ab}$	$11.2^{o\text{-}ab}$*$3.9^{v\text{-}ac}$	$60.6^{s\text{-}ad}$*$44.8^{n\text{-}x}$	$39.5^{e\text{-}h}$*$17.0^{h\text{-}k}$	$144.4^{i\text{-}m}$*95.1^{lm}
AS-14	$10.2_{v\text{-}ae}$*$4.2^{t\text{-}ac}$	41.8^{ad}*$29.4a^{a\text{-}ab}$	$11.7^{n\text{-}t}$*$4.3^{r\text{-}ab}$	$72.9^{o\text{-}y}$*$43.8^{o\text{-}z}$	$40.3^{c\text{-}e}$*$17.1^{g\text{-}k}$	$149.9^{i\text{-}l}$*$110.2^{i\text{-}k}$
AS-15	$10.4^{u\text{-}ae}$*$4.1^{t\text{-}ac}$	$80.8^{o\text{-}q}$*$44.9^{n\text{-}z}$	8.6^{af}*5.2^{q}	$73.6^{o\text{-}x}$*$41.9^{o\text{-}aa}$	$39.6^{d\text{-}h}$*$17.7^{c\text{-}h}$	$145.4^{i\text{-}m}$*$112.8^{h\text{-}k}$
AS-16	$10.5^{s\text{-}ae\ -ac}$*$3.8^{w\text{-}ac}$	$74.9_{o\text{-}w}$*$44.1^{o\text{-}z}$	$11.6^{n\text{-}u}$*$4.4^{r\text{-}aa}$	$75.5^{o\text{-}w}$*$51.9^{n\text{-}s}$	$41.1^{b\text{-}c}$*18.4^{bc}	$150.9^{i\text{-}k}$*94.9^{lm}
AS-17	$10.2^{v\text{-}ae}$*$4.1^{t\text{-}ac}$	$79.7^{o\text{-}s}$*$39.4^{q\text{-}ab}$	$11.8^{n\text{-}s}$*$5.0^{q\text{-}s}$	$80.0^{o\text{-}r}$*55.7^{no}	41.9^{b}*17.9c-e	$131.2^{l\text{-}n}$*95.7^{lm}
AS-18	$10.4^{t\text{-}ae}$*$4.0^{u\text{-}ac}$	$75.6^{o\text{-}w}$*$41.3^{p\text{-}ab}$	$9.8^{ab\text{-}af}$*$4.4^{r\text{-}aa}$	$62.2^{q\text{-}ad}$*$42.9^{o\text{-}aa}$	$38.2^{i\text{-}k}$*15.7^{mn}	284.3^{a}*196.6^{a}
AS-19	$11.8^{n\text{-}r}$*$4.2^{s\text{-}ac}$	$65.4^{p\text{-}ab}$*$41.9^{o\text{-}aa}$	$11.5^{n\text{-}v}$*$4.4^{r\text{-}aa}$	$73.6^{o\text{-}x}$*$49.7^{n\text{-}u}$	36.7^{lm}*13.8^{p}	$161.5^{g\text{-}i}$*$114.6^{g\text{-}j}$
AS-20	12.1^{no}*$4.4^{r\text{-}z}$	$72.2^{o\text{-}y}$*$43.1^{o\text{-}aa}$	12.7^{n}*5.0^{qr}	$73.8^{o\text{-}x}$*$49.4^{n\text{-}u}$	$39.5^{e\text{-}i}$*$17.1^{h\text{-}k}$	$154.5^{h\text{-}j}$*$113.9^{g\text{-}j}$
AS-21	$9.8^{ac\text{-}af}$*$3.8^{w\text{-}ac}$	$73.6^{o\text{-}x}$*$36.5^{u\text{-}ab}$	$9.9^{ab\text{-}af}$*$4.5^{q\text{-}z}$	$55.0^{z\text{-}ad}$*$35.2^{v\text{-}ab}$	$40.9^{b\text{-}d}$*$16.7^{j\text{-}l}$	$147.7^{i\text{-}m}$*$105.2^{j\text{-}l}$
AS-22	$10.1^{w\text{-}ae}$*$4.1^{t\text{-}ac}$	$67.8^{o\text{-}z}$*$33.9^{z\text{-}ab}$	$11.9^{n\text{-}q}$*$4.8^{q\text{-}t}$	$68.1^{o\text{-}z}$*$42.2^{o\text{-}aa}$	$37.5^{k\text{-}m}$*15.8^{mn}	$132.7^{k\text{-}n}$*$104.1^{j\text{-}l}$
AS-23	$10.0^{y\text{-}ae}$*$3.8^{x\text{-}ac}$	$57.2^{v\text{-}ad}$*$38.1^{s\text{-}ab}$	$11.3^{o\text{-}y}$*$4.7^{q\text{-}u}$	$67.1^{p\text{-}aa}$*$42.3^{o\text{-}aa}$	$39.7^{d\text{-}g}$*18.8^{b}	$146.9^{i\text{-}m}$*93.5^{lm}
AS-24	$10.1^{w\text{-}ae}$*$3.6^{aa\text{-}ac}$	$52.7^{z\text{-}ad}$*$32.6^{z\text{-}ab}$	$11.9^{n\text{-}p}$*$4.5^{q\text{-}z}$	$68.2^{o\text{-}z}$*$36.9^{t\text{-}ab}$	$37.5^{k\text{-}m}$*14.8^{o}	$150.2^{i\text{-}l}$*$106.5j^{j\text{-}l}$
AS-25	$10.1^{x\text{-}ae}$*$3.9^{v\text{-}ac}$	$56.7^{w\text{-}ad}$*$34.5^{w\text{-}b}$	$11.6^{n\text{-}u}$*$4.6^{q\text{-}x}$	$74.4^{o\text{-}w}$*$49.3^{n\text{-}u}$	$39.4^{e\text{-}i}$*$17.2^{e\text{-}j}$	$137.2^{j\text{-}m}$*$103.8^{j\text{-}l}$
AS-26	$11.6^{n\text{-}u}$*$4.0^{u\text{-}ac}$	$59.9^{t\text{-}ad}$*$37.3^{t\text{-}ab}$	$9.9^{aa\text{-}af}$*$4.0^{u\text{-}ac}$	$58.5^{u\text{-}ac}$*$40.4^{p\text{-}ab}$	$37.8^{j\text{-}l}$*$17.0^{h\text{-}k}$	$180.4^{e\text{-}g}$*$120.8^{f\text{-}i}$
AS-27	$10.6^{r\text{-}ae}$*$4.4^{r\text{-}aa}$	$65.1^{p\text{-}ab}$*$39.1^{r\text{-}ab}$	$10.7^{p\text{-}ae}$*$4.3^{r\text{-}ab}$	$62.1^{q\text{-}ac}$*$37.4^{t\text{-}ab}$	$39.7^{d\text{-}g}$*$18.2^{b\text{-}d}$	$151.4^{i\text{-}k}$*$106.1^{j\text{-}l}$
AS-28	$11.7^{n\text{-}u}$*$4.4^{r\text{-}aa}$	$66.8^{p\text{-}ab}$*$38.9^{r\text{-}ab}$	$11.1^{o\text{-}ab}$*$4.2^{t\text{-}ac}$	$76.3^{o\text{-}v}$*$49.6^{n\text{-}u}$	$40.1^{c\text{-}f}$*$16.3^{k\text{-}m}$	$186.0^{d\text{-}f}$*$132.4^{c\text{-}f}$
AS-29	$10.7^{p\text{-}ae}$*$3.9^{w\text{-}ac}$	$73.6^{o\text{-}x}$*$41.9^{o\text{-}aa}$	$11.1^{o\text{-}ac}$*$4.2^{s\text{-}ac}$	$75.2^{o\text{-}w}$*$49.4^{n\text{-}u}$	$39.1^{e\text{-}j}$*$17.6^{d\text{-}i}$	$151.6^{i\text{-}k}$*$102.0^{j\text{-}l}$
AS-30	$10.6^{q\text{-}ae}$*$4.0^{u\text{-}ac}$	$67.7^{o\text{-}z}$*$40.3^{q\text{-}ab}$	$11.4^{n\text{-}w}$*$4.4^{r\text{-}aa}$	$65.9^{p\text{-}ab}$*$37.1^{t\text{-}ab}$	$39.4^{e\text{-}i}$*$17.3^{e\text{-}j}$	173.9^{fg}*$114.5^{g\text{-}j}$
AS-31	$10.9^{o\text{-}ad}$*$4.0^{t\text{-}ac}$	$73.9^{o\text{-}x}$*$44.5^{n\text{-}z}$	$11.9^{n\text{-}p}$*$4.7^{q\text{-}v}$	$72.3^{o\text{-}y}$*$48.4^{n\text{-}w}$	$40.2^{c\text{-}f}$*$17.9^{c\text{-}g}$	175.1^{fg}*$110.1^{i\text{-}k}$
AS-32	$10.2^{v\text{-}ae}$*$3.9^{w\text{-}ac}$	$77.3^{o\text{-}u}$*$43.7^{o\text{-}z}$	$10.2^{v\text{-}ae}$*$4.6^{q\text{-}x}$	$81.1^{o\text{-}q}$*$47.2^{n\text{-}x}$	$39.0^{f\text{-}j}$*$17.1^{f\text{-}k}$	$180.5^{e\text{-}g}$*$121.5^{f\text{-}i}$
Mean	10.5^{C}*4.0^{C}	68.2^{B}*40.8^{C}	11.1^{B}*4.4^{B}	68.0^{B}*43.5^{B}	39.3^{A}*17.1^{A}	164.2^{A}*117.9^{A}

*** Each no. is a mean of three replicates**

**** Numbers followed by the same symbols significantly are not different at 0.05 level according to Duncan's multiple test analysis**

correlate with geographical origin, pathogenic variations, and disease development, as indicated in several previous studies (34). So, in the current study, the isolates were identified using a combination of different parameters and criteria (morphology, pathogenicity, and molecular) that resolved the identification, and as a result, 32 isolates were designated as *A. rabiei*. The differences between isolates may be important in the epidemiology of AB and may also affect the ability of isolates to create epiphytotic of the disease.

Effects of temperature on mycelial growth and colony characteristics of *A. rabiei* isolates: Mycelial growth average of *A. rabiei* isolates at various temperatures exhibited varying linear growth rates at temperatures ranges15-35°C (Table 4). The isolates showed radial mycelial growth at 15-30°C, and the growth ceased at 35°C after 20 days of incubation, even after 10 days of incubation at 25°C, all the tested isolates failed to resume growth. Mycelial growth rate increases as temperature increased up to 20°C (4.64cm), reached to maximum growth (7.35cm) at 25°C, then decreased as temperature increased and ceased at 35°C. Bedi and Aujla (9) found that at temperature higher than 31°C, growth continued but sporulation stopped on Richards agar medium. In general, isolates AS-11, AS-6, AS-32, AS-25, and AS-3 grew faster than other isolates, with no significant differences between them. While isolates AS-22, AS-19, AS-20, and AS-29 growth were slow. The current study clearly indicates the effect of temperature on mycelial growth of *A. rabiei* and demonstrate that different temperatures have significant effect on fungal mycelial growth. In general, the isolates grow well at temperature rang 20-25°C, and the best rapid mycelium growth occurred at 25°C. The isolates' mycelial growth rate slow below 15°C and above 30°C. When the temperature rises above 30°C, mycelial growth is terminated and completely stopped at 35°C. The discovered optimum temperature requirement for *A. rabiei* isolates growth was broadly consistent with previous research findings (40).

Table 4. Growth rate of different *Aschochyta rabiei* isolates collected from various chickpea fields in IKR on CSMDA at different temperatures after 20 days.

Isolate	Growth rate (cm)					
	15 °C	20 °C	25 °C	30 °C	35 °C	Mean
AS-1	2.40 aq-az	4.86 m-s	7.70 a-c	3.17 ac-ag	0 be	3.63 b-f
AS-2	2.43 ap-ay	4.70 n-x	7.35 c-j	3.16 ac-ah	0 be	3.53 d-h
AS-3	2.52 an-ax	5.12 m-o	7.43 b-i	3.19 ac-af	0 be	3.65 a-e
AS-4	2.18 aw-bc	4.98 m-q	7.35 c-j	2.77 af-ar	0 be	3.46 e-j
AS-5	2.22 av-bc	4.29 x-z	7.12 f-l	2.72 ai-at	0 be	3.27 j-m
AS-6	2.72 ai-at	5.03 m-p	7.99 a	3.30 ac-ae	0 be	3.81 ab
AS-7	2.33 as-az	4.38 u-z	7.27 c-k	3.11 ad-al	0 be	3.42 g-k
AS-8	2.67 aj-au	4.53 r-z	7.62 a-e	3.32 ab-ae	0 be	3.63 b-f
AS-9	2.12 ax-bd	4.11 z-aa	7.05 h-l	2.74 ag-at	0 be	3.20 lm
AS-10	2.12 ax-bd	4.45 s-z	7.08 f-l	2.75 ag-as	0 be	3.28 j-m
AS-11	2.72 ai-at	5.28 m	7.87 ab	3.30 ab-ae	0 be	3.83 a
AS-12	2.32 at-ba	4.33 w-z	7.38 c-i	2.70 ai-at	0 be	3.35 h-l
AS-13	2.43 ap-ay	4.36 v-z	7.42 c-i	3.05 ad-aj	0 be	3.45 f-j
AS-14	2.35 ar-az	4.49 r-z	7.20 d-k	2.55 am-ax	0 be	3.32 i-l
AS-15	2.23 ay-bb	4.68 o-x	7.33 c-j	2.82 af-aq	0 be	3.41 g-k
AS-16	2.50 an-ay	5.12 mn	7.47 b-h	2.85 af-ap	0 be	3.59 d-g
AS-17	1.98 az-bd	4.58 q-y	7.07 g-l	2.52 an-ax	0 be	3.23 k-m
AS-18	2.58 al-aw	4.68 o-x	7.62 a-e	3.03 ad-ak	0 be	3.58 d-g
AS-19	1.85 bb-bd	4.22 yz	6.73 l	2.77 af-ar	0 be	3.11 mn
AS-20	2.45 ao-ay	4.64 p-y	7.43 b-i	2.91 ad-an	0 be	3.49 d-i
AS-21	2.52 an-ax	4.97 m-q	7.52 b-f	2.95 ad-am	0 be	3.59 d-g
AS-22	1.73 bd	3.56 ab-ac	7.67 a-d	2.07 ay-bd	0 be	3.00 n
AS-23	2.13 ax-bd	4.48 s-z	7.02 i-l	2.71 ai-at	0 be	3.27 j-m
AS-24	1.78 bc-bd	4.83 n-t	6.83 kl	2.79 af-aq	0 be	3.25 k-m
AS-25	2.60 ak-aw	5.12 mn	7.50 b-g	3.17 ac-ah	0 be	3.68 a-d
AS-26	2.58 al-aw	4.74 n-w	7.29 c-j	3.00 ad-al	0 be	3.52 d-h
AS-27	2.18 aw-bc	4.74 n-w	7.43 b-i	2.88 ae-ao	0 be	3.45 f-j
AS-28	2.60 ak-aw	4.79 n-v	7.36 c-i	3.11 ad-al	0 be	3.57 d-g
AS-29	1.88 ba-bd	4.40 t-z	6.92 j-l	2.73 ah-at	0 be	3.19 l-n
AS-30	2.63 aj-av	4.92 m-r	7.35 c-j	3.13 ac-al	0 be	3.61 c-g
AS-31	2.73 ah-at	4.40 t-z	7.25 d-k	3.33 ab-ad	0 be	3.54 d-g
AS-32	2.83 af-aq	4.80 n-u	7.63 a-e	3.73 aa-ab	0 be	3.80 a-c
Mean	2.35d	4.64b	7.35a	2.95c	0.00e	3.46

Similar to our findings, Bahr et al. (7) reported that *A. rabiei* isolates grew at very slow rate after 25°C and the mycelial growth was completely terminated after 28°C. On the other hand, Ozkilinc et al. (33), reported that origin *A. rabiei* isolates grew at the same rates at 15°C and 25°C with faster grow at 25°C than 15°C on PDA. This is consistent with our findings, which show that the optimal mycelial growth temperature of *A. rabiei* is around 25°C. Ascochyta isolates may have different growth rates at different temperatures, such as *A. zeicola* isolated from maize exhibited optimal growth at 24.2°C, but the optimal growth of *A. stipae* and *A. ducisaprutii* from Antarctica was at 15°C (25). The current study confirms that temperature has a significant effect on *A. rabiei* mycelial growth rates. The optimum growth rate occurred at 25°C. This finding could help the future research on ABD and the natural behavior of *A. rabiei*. Such research could lead to abetter understanding of the conditions required for disease development, as well as improvement in disease control strategies. Furthermore, estimating the effects of environment factors on pathogens and their interactions in the field.

Phenotyping *of A. rabiei* isolates

Pathogenic variability results classified *A. rabiei* isolates into four groups (GA, GB, GC and GD) and 15 physiological races based on the isolates pathogenicity and virulence on a set of 10 chickpea differentials (Table 5). Race 1 characterized by its high aggressiveness and virulence against all the tested differentials. It was represented by 5 isolates (AS-1, AS-7, AS-9, AS-11 and AS-20) collected from Sulaimaniyha, Erbil and Garmian. Races 2, 3, and 4 were only avirulent on one genotype, FLIP07-197C, FLIP07-228C and Ghab-3 respectively. Race 2 and 3 are represented by a single Sulaimani isolate for each AS-23 and AS-3, respectively, whereas Race 4 represented by five isolates from Sulaimaniyha, Garmian and Erbil. Four races (5, 6, 7, 8 and 9) were found to be a virulent on two differentials (FLIP07-361C, FLIP09-388C; Ghab-3, FLIP09-388C; Ghab-3, FLIP09-222C; FLIP07-228C, FLIP09-248C; Ghab-3, FLIP09-229C) represented by isolates AS-4, AS-5, AS-12, AS-14 and AS-21 in Sulaimaniyha, Garmian and Erbil. Race 10 represented isolate AS-2 from Sulaimani was a virulent on three differentials, while Races 11 and 12 represented by AS-6 and AS-17 isolates from Sulaimani and Erbil were avirulent on 4 differentials each. Two races (13 and 14) represented by Halabja isolates AS-10 and AS-8 were weak and showed virulence on 3 differentials, whereas Race 15 represented by Erbil isolate AS-15 was a virulent and showed virulence only on FLIP09-197C (Table 5). Distribution of the supposed 15 *A. rabiei* races across different IKR provinces was quite variable. Pathogen diversity was high in Sulaimani, with nine different races (1, 2, 3, 4, 5, 6, 9, 10, and 11) discovered in various chickpea fields. This account for 60% of all races, followed by Erbil, which has five races (1, 4, 8, 12 and 15) and accounts for 33%. Halabja and Garmian each contributed three races (1, 13, 14) and two races (4, 7), accounting for 20% and 12% of the total. Races 4 and 5, each with 5 isolates, account for 43.47% of all the isolates and may be the largest and wide distributed races in Kurdistan region. The mean virulence of the tested isolates on ten differentials revealed four distinct virulence groups, GA- highly virulent, characterized by high level of virulence including five isolates (AS-7, AS-9, AS-19, AS-20, and AS-21) representing 21.73% of all isolates, GB- Virulent isolates included AS-1, AS-3, AS-5, AS-11, AS-12, AS-13, AS-16, AS-18, AS-22, and AS-23, representing the largest and most widely distributed group with 43.47%, GC- Moderately virulent, with moderate levels of virulence in AS-2, AS-4, AS-6, AS-14, and AS-17 (five isolates) representing 21.73%, and GD- A virulent, with low levels of virulence (13.04%) including three isolates AS-8, AS-10, and AS-15. Kaur, (24) found that *A. rabiei* isolates that grew quickly and produced little spores were less virulent than those produce a lot of spores. In other studies, no correlations were found between isolate virulence, geographical origin, and morphological characteristics such as spore size, colony color, and radial growth in vitro (2). Pathogenicity of various *A. rabiei* isolates in India was found to be highly correlated with the amount of phytotoxins produced (24). It would be interesting to investigate toxin production by

various Kurdistan isolates and see if there is a link to aggressiveness. The existence of different races of *A. rabiei* is suspected due to differences in host-pathogen interaction and breakdown of host resistance in some cultivars (23). Mutation is thought to have evolved aggressive isolates in response to a change in host resistance. Pathogenic variability in *A. rabiei* has been reported from many countries, including India (5), Syria and Lebanon (43), and United States (20). These studies were based on 3 to 15 chickpea differentials tested with 11-130 *A. rabiei* isolates and classified into 3 to 14 races.

Table 5. Pathogenicity and virulence spectrum of twenty-three *Aschochyta rabiei* isolates collected from major chickpea growing area across Iraq under artificial inoculation conditions under control conditions

Isolate	Genotypes										Mean	Supposed Race
	FLIP07 - 197C	ILC 263	Ghab3	FLIP07 -228C	FLIP09 -222C	FLIP09 -229C	FLIP09 -248C	FLIP09 -384C	FLIP07 -361C	FLIP09 - 388C		
AS-1	V^{a-z}	V^{a-q}	V^{a-h}	V^{a-y}	V^{a-aa}	V^{a-ac}	V^{a-v}	V^{a-t}	V^{a-z}	V^{a-p}	V^{a-c}	1
AS-2	V^{a-x}	V^{a-v}	Av^{m-al}	V^{c-al}	V^{a-z}	V^{a-ac}	V^{a-ae}	Av^{f-al}	Av^{a-s}	V^{c-al}	MV^{c-f}	10
AS-3	V^{a-w}	V^{a-v}	V^{a-x}	Av^{k-al}	V^{c-al}	V^{a-p}	V^{b-af}	V^{a-s}	V^{a-x}	V^{b-af}	V^{b-e}	3
AS-4	V^{a-aa}	V^{a-z}	V^{a-x}	V^{b-ae}	V^{a-ac}	V^{a-t}	V^{a-z}	V^{a-ac}	Av^{g-al}	Av^{l-al}	MV^{c-f}	5
AS-5	V^{a-x}	V^{a-w}	Av^{j-al}	V^{a-aa}	V^{a-x}	V^{b-ae}	V^{a-t}	V^{a-w}	V^{a-z}	Av^{g-al}	V^{b-e}	6
AS-6	Av^{m-al}	Av^{e-al}	Av^{k-al}	Av^{g-al}	V^{a-z}	V^{a-z}	V^{a-w}	V^{a-ab}	V^{a-x}	V^{c-ag}	MV^{d-f}	11
AS-7	V^{a-x}	V^{a-r}	V^{a-x}	V^{a-ab}	V^{a-q}	V^{a-u}	V^{a-n}	V^{a-n}	V^{a-v}	V^{a-r}	HV^{ab}	1
AS-8	Av^{m-al}	V^{c-ah}	Av^{ae-al}	Av^{v-al}	V^{c-al}	V^{c-al}	Av^{s-al}	Av^{q-al}	Av^{q-al}	Av^{x-al}	Av^{g}	14
AS-9	V^{a-n}	V^{a-k}	V^{c-al}	V^{a-l}	V^{a-k}	V^{a-c}	V^{a-q}	V^{a-q}	V^{a-f}	V^{a-x}	HV^{a}	1
AS-10	Av^{k-al}	Av^{r-al}	V^{a-aa}	Av^{w-al}	Av^{f-al}	V^{a-x}	Av^{h-al}	Av^{k-al}	V^{a-x}	Av^{g-al}	Av^{fg}	13
AS-11	V^{a-l}	V^{a-ad}	V^{a-ad}	V^{a-z}	V^{a-aa}	V^{a-ad}	V^{c-al}	V^{a-x}	V^{d-al}	V^{a-r}	V^{b-e}	1
AS-12	V^{c-al}	V^{a-y}	Av^{f-al}	V^{a-y}	Av^{j-al}	V^{a-x}	V^{a-y}	V^{a-x}	V^{a-x}	V^{a-q}	V^{b-f}	7
AS-13	V^{a-ac}	V^{b-af}	Av^{f-al}	V^{b-af}	V^{a-z}	V^{a-q}	V^{a-aa}	V^{a-ad}	V^{a-x}	V^{a-o}	V^{b-e}	4
AS-14	V^{c-al}	V^{a-ac}	V^{c-al}	Av^{f-al}	V^{f-al}	V^{a-t}	Av^{k-al}	V^{a-x}	V^{a-y}	V^{a-j}	MV^{c-f}	8
AS-15	V^{b-ae}	Av^{o-al}	Av^{z-al}	Av^{k-al}	Av^{l-al}	Av^{p-al}	Av^{p-al}	Av^{f-al}	Av^{n-al}	Av^{j-al}	Av^{g}	15
AS-16	V^{a-x}	V^{a-aa}	Av^{r-al}	V^{a-t}	V^{a-z}	V^{a-w}	V^{c-al}	V^{a-ac}	V^{a-v}	V^{a-ad}	V^{b-f}	4
AS-17	V^{a-ae}	V^{a-aa}	Av^{e-al}	Av^{k-al}	Av^{f-al}	V^{c-al}	Av^{i-al}	V^{c-ag}	V^{a-v}	V^{a-ad}	MV^{ef}	12
AS-18	V^{a}	V^{a-x}	Av^{a-ae}	V^{a-n}	V^{a-u}	V^{a-x}	V^{a-ad}	V^{b-ae}	V^{a-v}	V^{a-q}	V^{a-c}	4
AS-19	V^{a-v}	V^{a-i}	Av^{k-al}	V^{a-l}	V^{a-q}	V^{a-t}	V^{a-j}	V^{a-t}	V^{a-f}	V^{a-i}	HV^{a}	4
AS-20	V^{a-l}	V^{a-d}	V^{c-af}	V^{a-k}	V^{a-v}	V^{a-t}	V^{a-I}	V^{a-g}	V^{a-v}	V^{a-i}	HV^{a}	1
AS-21	V^{a-e}	V^{ab}	Av^{h-al}	V^{a-I}	V^{a-p}	Av^{i-al}	V^{a-l}	V^{a-i}	V^{a}	V^{a-v}	HV^{a}	9
AS-22	V^{b-af}	V^{a-w}	Av^{t-al}	V^{a-r}	V^{a-v}	V^{a-o}	V^{a-m}	V^{a-t}	V^{a-v}	V^{a-x}	V^{a-d}	4
AS-23	Av^{u-al}	V^{a-w}	V^{a-t}	V^{a-t}	V^{a-u}	V^{a-v}	V^{a-v}	V^{a-z}	V^{a-u}	V^{a-h}	V^{a-c}	2
Control	Av^{ag-al}	Av^{ah-al}	Av^{y-al}	Av^{aa-al}	Av^{ad-al}	Av^{af-al}	Av^{ab-al}	Av^{ah-al}	Av^{ac-al}	Av^{al}	Av^{h}	-
Mean	MS^{a}	S^{a}	T^{b}	MS^{a}	MS^{a}	S^{a}	S^{a}	S^{a}	S^{a}	S^{a}	S	

*** Av= Avirulent (1-40 % severity), MV= Virulent (41-50% severity), V= Virulent (51-60% Severity), HV= Highly virulent (61-100%**

**** T= Tolerant (16-40% Severity), MS= Moderately Susceptible (41-50% Severity), S= Susceptible (51-75% Severity).**

Udupa and Weigand (43) suggested that standard set of 3 chickpea differentials is sufficient in pathotyping *A. rabiei* isolates into 3 pathotypes based on increasing level of aggressiveness. Reddy and Kabbabeh (41) proposed a set of 6 differential genotypes to determine 6 physiological races. *A. rabiei* pathotypes were obtained using 130 and 64 isolates from Pakistan and Turkey, respectively (59). Udupa et al. (43) found 5 isolates from pathotype II in Syria. All the 6 physiological races of *A. rabiei* were found by Reddy and Kabbabeh (41) using 64 isolates from Syria and Lebanon. Using the same set Dolar and Gürcan (15) reported races 1, 4 and 6 in Turkey. all the 6 races reported in Turkey in 2009 (42). Chen et al. (12) reported that all the 5 races except race 6 are pathotype I. Chickpea cultivar ILC 3279 which is source of resistance to ABD and shows high level of resistance in several countries have been identified to be susceptible to race 6 (28), Thus, pathotype III was designated to both race 5 and race 6. A new highly aggressive pathotype known as IV was discovered in Syria (20). Currently, almost all studies around the world use pathotype rather than race to determine the virulence of isolates. It is difficult to study the pathogen's pathogenic variability and compare it to other researcher's findings because they used different methods and chickpea genotypes (12).There had been no previous studies on physiological characterization of *A. rabiei* isolates in Iraq particularly in Kurdistan region, with the exception of Al-Taee study (4), which identified five *A. rabiei* races namely A, B, C, D and H in Nineveh province. The current study is the first in Iraq to classify twenty-three *A. rabiei* isolates collected from various chickpea fields in Iraqi Kurdistan region into four groups and 15 physiological races.

Analysis of ITS sequences

The ITS 1 and ITS 4 primers amplified a 541bp band in all *A. rabiei* isolates (Figure 3). The sequencing results of 35 representative isolates confirmed identification of the fungal pathogen as *A. rabiei* with 100% nucleotide sequence similarity to the ITS region sequences available on the National Center for Biotechnology Information (NCBI). The number of nucleotide sequences, on the other hand ranged from 481 to 541bp. The shortest sequence (361bp) was found in AS-29, followed by (421bp) in AS-20 and AS-35, and the longest (541bp) was found in Isolates AS-2, AS-5, AS-6, AS-8, AS-10, AS-11, AS-13, AS-15, AS-16, AS-19, AS-24, AS-28, AS-30, AS-31, AS-32, AS-33, and AS-34, while other isolates such as AS-1, AS-3, AS-4, AS-7, AS-9, AS-12, AS-14, AS-17, AS-18, AS-21, AS-22, AS-23, AS-25, AS-26, and AS27 characterized with 481bp. The ITS sequences of all the isolates were registered at NCBI Gen Bank under different accession numbers (Table 6). Amplification of nuclear ITS sequences resulted in a single band in each accession (Figure 3). The bootstrap values of these sequences were low. This is because the polymorphism caused by sequence alignment is a single nucleotide polymorphism. The out-group species *Alternaria arborescens* was well resolved from the target sequences *A. rabie*i.

Phylogenic analysis

The phylogenetic tree built using the ITS sequences generated in this study grouped of all the isolates into a single cluster (Figure 4). *A. rabiei* isolates were highly similar, with 100% similarity. The phylogenetic tree which was contricted using the sequences of the representative isolates sequences as well as six ITS sequences from other countries submitted to NCBI GenBank, showed that the six international isolates from Argentina, Germany and India were clustered in the same group as our isolates (Figure 4). All the isolates grouped together showed high level of similarity (100%) in term of the pathogen ITS region. The phylogenetic tree clearly indicates that isolates originated from the same district can be partially clustered together and share high degree of similarity. Despite their high similarity, the tested isolates had little variation in the number of ITS sequences (481-541bp), which could be attributed to variations in the specific genotypes cultivated area as well as variations in environmental conditions, causing pathogen populations to adopt variability. Previous findings support the current findings which refer to the close relationship and high level of similarity of ITS1-5.8S-ITS2 rDNA regions in *A. rabiei* isolate sequences (8).

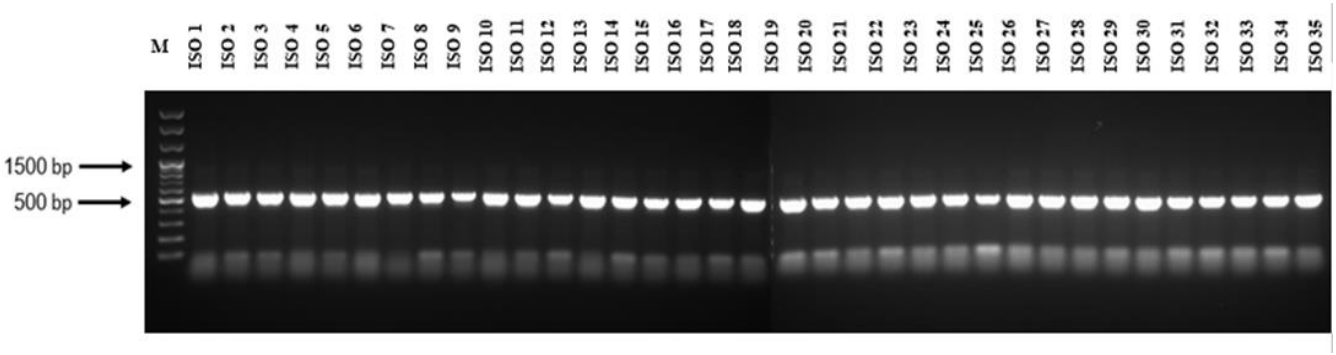

Figure 3. Agarose gel electrophoresis of PCR amplified ITS region of *Ascochyta rabiei* isolates, locations and isolate number. Iso. 1 to 35 represents: Bngrd/l, Khrabeh/2, Greza/3, Saraw/4, Kani speka/5, Mowan/6, Bershka/7, Basharty khwaraw/8, Hana zhalla/9, Banishar/10, Gula khana/11, Ganma rash/12, Bawa Nur/13, Afryan/14, Batas/15, Bawyan/16, Sisawa/17, ShawrAwa/18, Shewa Rash/19, Belangry Khwaraw/20, Grdjan/21, Kani Maran/22, Sultanade/23, Sarwchawa/24, Dwawa/25, Shkarta/26, Badawan/27, Shamsawa/28, Wazha/29, Kani waysa/30, Bakrajo/31, Bakrajo/32, Bizeniyan/33, Qaymasa/34, Khewata/35, and M-100bp DNA ladder

Table 6. Accession numbers of *A. rabiei* isolates from different chickpea fields identified using ITS1 and ITS4 sequences.

Isolate	Fungal Identified	Accession Numbers	Query Cover %	Identic Number %	Accession No. of Blast Identification	Country Identification
AS-1	*Ascochyta rabiei*	MZ323178				
AS-2	*Ascochyta rabiei*	MZ323179				
AS-3	*Ascochyta rabiei*	MZ323180	100	99.26	KU948513	Argentina
AS-4	*Ascochyta rabiei*	MZ323181				
AS-5	*Ascochyta rabiei*	MZ323182				
AS-6	*Ascochyta rabiei*	MZ323183				
AS-7	*Ascochyta rabiei*	MZ329151				
AS-8	*Ascochyta rabiei*	MZ323184	100	98.37	MT252609	India
AS-9	*Ascochyta rabiei*	MZ323185				
AS-10	*Ascochyta rabiei*	MZ323186				
AS-11	*Ascochyta rabiei*	MZ314597				
AS-12	*Ascochyta rabiei*	MZ314598				
AS-13	*Ascochyta rabiei*	MZ314599	100	99.56	EU167600.1	Germany
AS-14	*Ascochyta rabiei*	MZ314600				
AS-15	*Ascochyta rabiei*	MZ314601				
AS-16	*Ascochyta rabiei*	MZ314602				
AS-17	*Ascochyta rabiei*	MZ314603				
AS-18	*Ascochyta rabiei*	MZ314604	100	99.36	MT252615	India
AS-19	*Ascochyta rabiei*	MZ314605				
AS-20	*Ascochyta rabiei*	MZ314606				
AS-21	*Ascochyta rabiei*	MZ323092				
AS-22	*Ascochyta rabiei*	MZ323093	100	98.71	KT962083.1	India
AS-23	*Ascochyta rabiei*	MZ323094				
AS-24	*Ascochyta rabiei*	MZ323095				
AS-25	*Ascochyta rabiei*	MZ323096				
AS-26	*Ascochyta rabiei*	MZ323097				
AS-27	*Ascochyta rabiei*	MZ323098				
AS-28	*Ascochyta rabiei*	MZ323099				
AS-29	*Ascochyta rabiei*	MZ323100				
AS-30	*Ascochyta rabiei*	MZ323101	100	98.37	MT252609	India
AS-31	*Ascochyta rabiei*	MZ323102				
AS-32	*Ascochyta rabiei*	MZ323103				
AS-33	*Ascochyta rabiei*	MZ323104				
AS-34	*Ascochyta rabiei*	MZ323105				
AS-35	*Ascochyta rabiei*	MZ323106				

The use of sequence information from rDNA repeat units is well established in fungal taxonomy for characterizing isolates and resolving taxonomic ambiguities and isolate definition as this gene cluster occurs within chromosomes with multiple copies in a single nucleus (3). These rDNA arrays have been homogenized by evolution, and the functional nature of these gene blocks minimizes mutation within sequences, making them useful in taxonomy (38). As a result, a nucleotide sequence comparison of the ITS region of the tested *Ascochyta* isolatess with other sequences available online in NCBI gene bank confirmed the isolates identity as *A. rabiei*.

Conclusions. The current study indicates that AB is a significant disease in various chickpea production areas in Iraqi Kurdistan region. Cultural, macroscopic and microscopic differences between *A. rabiei* isolates were found to be significant. Fifteen races were identified by phenotyping all *A. rabiei* isolates on 10 differentials. Pathogen diversity was high in Sulaimani, where 9 different races were found in various chickpea fields, followed by Erbil, which had five races. The ITS sequences classified all the races into three groups within one cluster, which were all registered at NCBI Gen Bank under different accession numbers. This is, to the best of our knowledge, the first report in Iraq on the full identification of *A. rabiei* isolates using morphological and molecular methods.

Figure 4. Phylogenetic tree generated from the ITS sequences of *Ascochyta rabiei* isolates and other International isolates using MEGA X software and neighbor-joining methods with bootstrap values (1000 replicates).

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