First Report of Alternaria Alternata Causing Brown Leaf Spot on Apricot (Prunus Armeniaca) in Karbala Province of Iraq

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Abstract

During a survey in season 018, leaf spot symptoms were commonly observed on apricot (Prunus armeniaca) trees in the orchards of Al-Hussainiya district in Karbala Province of Iraq. The symptomatic leaves were gathered, the associated fungus was isolated and characterized relied on its morphological features and ITS-rDNA sequencing. The causative factor was found to be the fungus Alternaria alternata that caused distinguishable leaf spot symptoms on the inoculated leaves of apricot. Based on a review of previous references related to this disease in Iraq, this is the first report of the brown leaf spot disease caused by A. alternata in Karbala province of Iraq.

Keywords: Alternaria, Armeniaca, Karbala, Spot.

1. Introduction

Apricot (Prunus armeniacaL., family Rosaceae) is native to China [1]. Currently, it has been widely cultivated in the most of world including Iraq because of its multiple benefits. For example, the early harvesting season, pleasant taste, high nutritional value including the high quantity of β-carotene [2,3] that is considered the main precursor of vitamin A. In addition, the apricot fruits contain some pharmacological important compounds such as the high antioxidant content. Furthermore, the β-carotene of apricot is supposed to be valuable for inhibiting or decreasing the general weakness affected by oxidative stress, nephrotoxicity and methotrexate-induced intestinal damage [4]. Recently, the production of apricot trees worldwide has subjected the serious damage from different diseases caused by various pathogens [5].

There are more than three hundred species of Alternaria genus that are either saprotrophic in a wide range of environments or pathogenic affecting a diversity of plant hosts [6,7]. The accurate and quick identification of these species has become more difficult due to high morphological similarity among them [8,9]. Therefore, the molecular analysis combining with the morphological features provide the viable practice of the precise and rapid identification of the species of this genus and others [10-9].

One of the most common species of Alternariagenus is A. alternata that was reported producing several toxins such as alternariol (AOH), alteratoxin II (AXT II), and alternariol monomethyl ether (AME) [12]. Furthermore, it causes different diseases including the leaf spot on various plant hosts in different countries such as on Farfugium japonicum in China [13], Chili (Capsicum annuum L.) in Pakistan [14] and on Anemone japonica in Italy [15]. However, the A.alternata fungus has not been reported as a pathogen of apricot leaf spot in Iraq yet, especially in Kerbala provinces. As a result of the widespread leaf spot disease on various fruit trees, including apricot in the orchards of Al-Hussainiya district of Karbala province during growth season of 2018, a survey was conducted in this study to identify accurately the causes of this disease.

2. Materials and Methods

2.1. Morphological characterization

Leaves of apricot trees showing leaf spot symptoms were gathered randomly and excised into 0.5–1 cm long section, sterilized in 2% sodium hypochlorite for 3 min and washed thoroughly in sterile distilled water. These sterilized sections were laid on water agar medium and incubated for 3 days at 25 °C. A hypha tip technique was applied to purify the fungal
colonies. Each colony was purified on potato dextrose agar medium (PDA) and incubated at 25 ± 2°C for one week. The cultural and microscopic features of isolated fungus were examined and compared with previous descriptions [8].

2.2. Molecular identification

The whole genomic DNA was extracted from the one-week-old representative fungal isolate utilizing the DNeasy Plant Mini Kit. The pair universal primers (ITS1+ITS4) were used to amplify the internal transcribed spacer (ITS) region of the fungal ribosomal DNA [16]. The PCR products were sent to Macrogen company (Seoul, South Korea) for sequencing. The DNA sequence obtained was compared with fungi data available at NCBI-GenBank database using Basic Local Alignment Search Tool (BLAST) platform. The phylogenetic analysis was accomplished by Molecular Evolutionary Genetics Analysis (MEGA) program [17]. Finally, the identified sequence was deposited at GenBank database with an exclusive accession number.

2.3. The pathogenicity test

The pathogenicity of the representative fungal isolate was evaluated exploiting a detached leaf assay [18]. In brief, healthy apricot leaves were collected and sterilized in 2% sodium hypochlorite solution. The disinfected leaves were located on kitchen paper tissue in a plastic container. A conidial suspension of the fungus at concentration 1 × 10^6 conidia/mL was applied equally on the disinfected leaves (1 ml/leaf). However, control leaves were covered with sterilized distilled water only. Subsequently, all the inoculated and non-inoculated apricot leaves were incubated at 25 °C for one week. Three replicates (six leaves /replicate) were accomplished for each treatment.

3. Results and Discussion

3.1. The morphological and molecular characterization

The fungus associated with apricot leaves displayed leaf spot symptoms (Fig. 1a) was isolated. The growth of fungus was rapid on PDA media developing mycelia in blackish green to olive color colonies (Fig. 1b). The conidia (Fig. 1c) produced have 3-5 crosswise and some longitudinal septa. The conidia shape was obclavate possessing a short conical beak. The conidiophores were singly or some time in branches. These morphological characters were similar to previous descriptions mentioned earlier in this study[19,20]. Accordingly, the fungus was identified as specie of Alternaria genus.

The ITS-rDNA region successfully amplified and PCR products were sequenced and deposited into the NCBI-GenBank database under the accession number MK070043.1. The BLAST analysis showed 98-99% similarity with several global strains of A. alternata species. Phylogenetic analysis displayed grouping of the fungus strains obtained in this study with several international strains of A.alternata in the same clade particularly those with accession numbers MK764939.1 and KJ466978.1 (Figure 2).
3.2. The pathogenicity test

The disease symptoms were observed on the inoculated leaves of apricot as small brown spots, which grow into irregular dark brown spots and occasionally progress to be a target-like pattern of rings. However, the control leaves were asymptomatic. The A. alternata was re-isolated from the diseased leaves. The entire results of this study proved that the A. alternata fungus is the causal agent of the apricot leaf spot in Karbala province, Iraq. This fungus has not been reported previously in Iraq as a pathogen of this disease on apricot trees, hence, this is the first report of the disease on apricot (P. armeniaca) in Iraq.

Conclusions

In the current study, A. alternata was identified for the first time as a pathogen of apricot leaf spot in Karbala province, Iraq. This identification was based on the morphological, molecular and pathogenic characteristics of the pathogen.
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