Mycopath (2018) 16(1): 7-10 **Research Article**

Alternaria gaisen: A new pathogen causing leaf spot of *Gerbera jamesonii* from Pakistan

Shagufta Perveen, *Naureen Akhtar and Madiha Nayab

Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan.
**Corresponding author's email: naureen.iags@pu.edu.pk*

Abstract

Typical symptoms of *Alternaria* leaf necrosis were observed on leaves of gerbera daisy (*Gerbera jamesonii*. L) growing in a garden in Lahore, Pakistan. Pathogen was isolated from the infected leaf tissues and identified on morphological as well as molecular characteristics by nucleotide sequencing of internal transcribed spacer (ITS) region of rDNA. Isolated fungus was identified as *Alternaria gaisen*. The fungus was further differentiated from closely related species of genus *Alternaria* by phylogenetic analysis. Pathogenicity of the isolated pathogen was verified following Koch's pathogenicity postulates. *Alternaria* leaf necrosis caused by *A. gaisen* is reported for the first time from Pakistan.

Keywords: *Alternaria*, Fungus, Internal Transcribed Spacer, Necrosis, Pathogenicity.

Introduction

Genus *Alternaria* includes both plant pathogenic and saprophytic species. Pathogenic species may cause leaf necrosis and blights on a variety of host plants (Bashir *et al.,* 2014; Akhtar *et al.,* 2016). *Gerbera jamesonii* Bolus ex. Hook is an important horticultural crop due to its beautiful and colorful flowers. This plant is commercially cultivated throughout the world. Little information is available about the *Alternaria* diseases of *G. jamesonii* worldwide. *Alternaria gerberae*, *A. alternata*, *A. porri*, *A. solani* and *A. dauci* are some of the reported leaf necrotic pathogens of gerbera (Kulibaba, 1972; Mirkova and Konstantinova, 2003).

Identification and differentiation of most of the species of *Alternaria* is quite complex due to huge morphological plasticity when grown under variable conditions of temperature, type of medium and photoperiod (Simmons, 2007). To overcome this problem, polyphasic taxonomic approach in which morphological characters combined with DNA sequence analysis is employed (Woudenberg *et al.,* 2013). rDNA sequence analysis of conservative internal transcribed spacer (ITS) region has been used successfully for the rapid and reliable identification of several species of *Alternaria* (Wang *et al.,* 2001; Konstantinova *et al.,* 2002; Pryor and Michailides, 2002; Akhtar *et al.,* 2015). Objectives of the present research were to identify new leaf spot disease of *G. jamesonii* caused by *Alternaria* species from Pakistan and to perform phylogenetic analysis of species to find out the evolutionary relatedness of fungal pathogen with similar species.

Materials and Methods

Sampling, symptoms study and pathogen isolation

Disease samples were collected during October/November, 2015 from Lahore Pakistan. Leaves of *G. jamesonii* were found to be infected with small brown circular spots of 1 to 3 mm size. Almost fifty plants were present at the sampling site and all exhibited the similar disease symptoms. On an average, 30% leaf area was found to be infected. Diseased leaves were sampled randomly from ten different plants and brought to the laboratory in sterilized polythene bags for pathogen study. For the isolation of pathogen, four infected leaves per plant and three necrotic spot per leaf were selected. Each selected necrotic spot was cut into 3 mm^2 pieces, surface disinfected by sodium hypochlorite and inoculated onto malt extract agar (MEA) medium. Petri plates were incubated at 25 $\pm$ 2 °C for 2 to 3 days. For purification of culture, hyphal tips were sub-cultured and allowed to grow at 25 $\pm$ 2 °C.

Pathogen identification and phylogeny

Phenotypic observations were made on seven days old pure culture. Complete description of macro- and micro-morphological characters was prepared to key out the species using authentic published literature (Simmons, 2007). Micrometry and microphotography were also carried out. For the confirmation of morphology based identification, amplification of internal transcribed spacer (ITS) region of rDNA was carried out using total genomic DNA as template and universal primer pair ITS1 forward and ITS4 reverse (White *et al.,* 1990). PCR reaction was carried out in thermocycler in a 25 µL PCR reaction mixture as described by Akhtar *et al.* (2014a). Amplified gene product was sent for nucleotide sequencing and resulting DNA sequence was analyzed using bioinformational tools.

Evolutionary analyses were conducted in MEGA6 (Tamura *et al.,* 2013) using 24 nucleotide sequences from different species of *Alternaria*. Phylogenetic tree was constructed using UPMGA

(Sneath and Sokal, 1973). The evolutionary distances were inferred by applying Maximum Composite Likelihood method (Tamura *et al.,* 2004).

Verification of Koch's pathogenicity postulates

For the confirmation of pathogenicity of the isolated fungus, soil of three gerbera plants growing in separate pots, was inoculated with the 5×10^4 spores of pathogen while similar three plants were treated with water to act as control. Plants were kept in a glass house at 25 $\pm$ 2 °C, watered regularly and monitored for the emergence of disease symptoms.

Results and Discussion

Morphological characters revealed that fungal colony was fast growing on MEA reaching 5.5 to 6 cm in diameter in 7 days. The front color of colony was black (Fig. 1B), while reverse was dark brown to blackish (Fig. 1C). No pigmentation and zonation was observed in culture. The conidia were arranged in the form of short chains that every chain has four to six conidia (Fig. 1D). *Conidiophores* were pale brown and 3.5 µm thick in diameter. C*onidia* (Fig. 1E) were blunt tapper to ovoid and olive brown in color. The conidial origination was monoblastic. Some conidia were geniculate and some were not. Some conidia were medium sized and range in size 9 - 10 x 11 - 26 µm while some were larger in size of 10 - 13 x 31 - 37 µm. Pathogen was identified as *Alternaria gaisen* (Simmons, 2007; Akhtar *et al.,* 2014b). Pathogenicity test result showed the similar disease symptoms after fifteen days of inoculation. However, control plants remained asymptomatic. Re-isolation of same pathogen from artificially inoculated plants confirmed the Koch's pathogenicity postulates. An agar slant of pathogen culture was deposited to FCBP under the accession number of FCBP1510. Differentiation between *A. alternata* and *A. gaisen* is difficult on the basis of morphology. To overcome this problem, molecular characterization is recommended (Woudenberg, 2015).

Resulting nucleotide sequence of amplified ITS region was BLAST and percentage homology of this strain was obtained with respective *A. gaisen* strains in GenBank. *Alternaria gaisen* (FCBP1510) showed 100% similarity with *A. gaisen* strains deposited under the accession numbers KU293580, EU520078, AF314574 and KP638337 while 99% to many of its respective strains. This sequence was submitted to GenBank under the specific accession number KT283674. Phylogenetic tree constructed using sequences from twenty-three different species along with *A. gaisen* isolated in present study, delimited this strain from rest of the strains (Figure 2). The nucleotide sequences along with their accession numbers of the strains compared for present investigation were retrieved from the GenBank.

Genomic sequencing has revolutionized the field of fungal taxonomy as this is a more accurate and easy method and need less expertise. Nucleotide sequencing of ITS region is used successfully to characterize the fungal species including members of genus *Alternaria* (Bashir *et al.,* 2014; Shoaib *et al.,* 2014). Similarly, in present study both morphological and molecular approaches are used for reliable identification of pathogen.

Gerbera is an important horticultural plant, this disease need special attention with reference to its spread and economic loss as well as management to reduce the losses by this emerging pathogen.

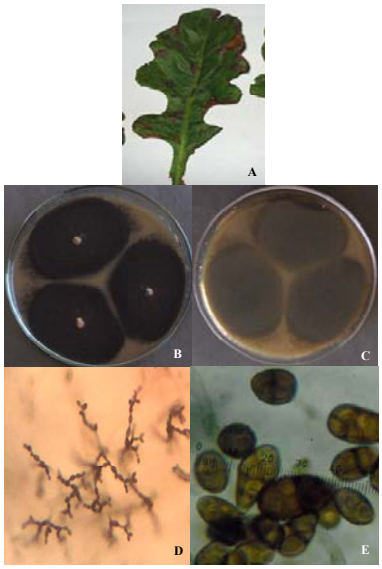

Fig. 1. *Alternaria gaisen* (FCBP1510). **A:** An infected leaf of *G. jamesonii* showing symptoms of necrosis. **B:** Front side of colonies; **C:** Reverse colonies; **D:** Conidia arranged in the form of chains under stereoscope, **E:** Conidia at 100X magnification of microscope.

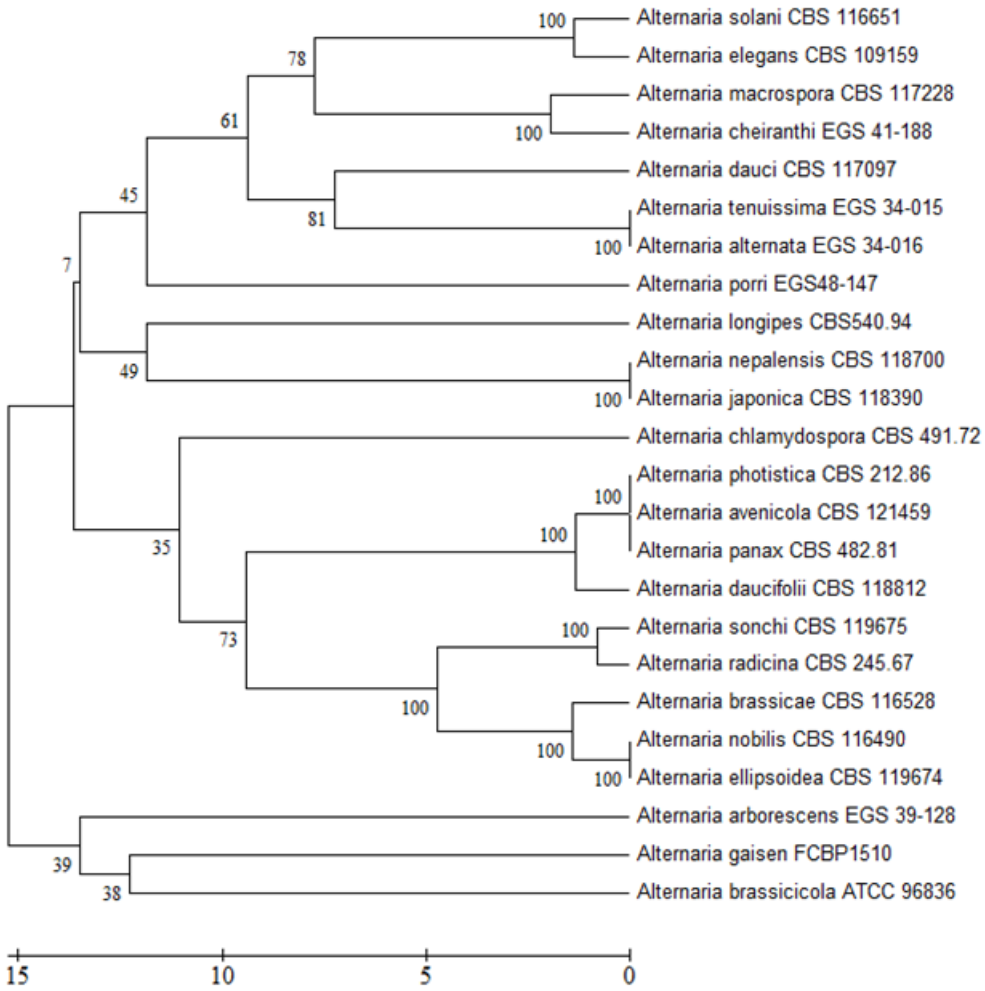

Fig. 2. Evolutionary relationships of different species of genus *Alternaria* inferred using the UPGMA method. The percentage of replicate trees in 500 bootstrap replicates are written beside each branch (Felsenstein, 1985). The tree is drawn to scale which shows the evolutionary distances used to infer the phylogenetic tree.

References

Akhtar N, Hafeez R, Awan ZA, 2014b. First report of rice leaf spot by *Alternaria gaisen* from Pakistan. *Plant Dis.,* **98**: 1440.

Akhtar N, Naseer A, Shafique S, Shafique S Nayab M, 2015. First report of *Alternaria arborescens* causing leaf spot of *Dracaena mariginata* in Pakistan. *J. Plant Pathol.,* **97**: S70.

Akhtar N, Nayab M., Sattar N, Qurashi F, 2016. First report of *Alternaria alternata* causing leaf spot of *Polyalthia longifolia* in Pakistan. *J. Plant Pathol.*, **98**: 173.

Akhtar N, Shoaib A, Munir S, Ali A, Khurshid S, 2014a. Isolation, identification and enzyme production profile of *Aspergillus niger*. *J. Anim. Plant Sci.,* **24**: 1438-1443.

Bashir U, Mushtaq S, Akhtar N, 2014. First report of *Alternaria metachromatica* from Pakistan causing leaf spot of tomato. *Pak. J. Agric. Sci*., **51**: 315-318.

Bhat HA, K. Ahmad RA, Ahanger N.A, Qazi NA, Dar Ganie SA, 2013. Status and symptomatology of *Alternaria* leaf blight (*Alternaria alternata*) of Gerbera (*Gerbera jamisonii*) in Kashmir valley. *Afr. J. Agric. Res.,* **8**: 819-823.

Farhood S, Hadian S, 2012. First report of Alternaria leaf spot on Gerbera (*Gerbera Jamesonii* L.) in North of Iran. *Adv. Environ. Biol*., **6**: 621-624.

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

Konstantinova P, Bonants PJM, van Gent-Pelzer M, van der Zouwen P, van den Bulk R, 2002. Development of specific primers for detection and identification of *Alternaria* spp. in carrot material by PCR and comparison with blotter and plating assays. *Mycol. Res.*, **106**: 23-33.

Kulibaba UF, 1972. Diseases of Gerbera grown under cover. *Gl. Bot. Sad. Byul.*, **83**: 115-119.

Mirkova E, Konstantinova P, 2003. First report of *Alternaria* leaf spot on Gerbera (*Gerbera*

jamesonii H. Bolux ex J. D. Hook) in Bulgaria. *J. Phytopathol.,* **151**: 323-328.

Nagrale DT, Gaikwad AP, Sharma L, 2013. Morphological and cultural characterization of *Alternaria alternata* (Fr.) Keissler blight of gerbera (*Gerbera jamesonii* H. Bolus ex J. Hook). *J. Appl. Nat. Sci.*, **5**: 171-178.

Pryor BM, Michailides TJ, 2002. Morphological, pathogenic, and molecular characterization of *Alternaria* isolates associated with *Alternaria* late blight of pistachio. *Phytopathology,* **92**: 406-416.

Shoaib A, Akhtar N, Akhtar S, Hafeez R, 2014. First report of *Alternaria longipes* causing leaf spot of potato cultivar Sante in Pakistan, *Pl. Dis.*, **98**: 1742.

Simmons EG, 2007. *Alternaria*: An Identification Manual. CBS, Fungal Biodiversity Center Utrecht, The Netherlands.

Sneath PHA, Sokal RR, 1973. Numerical Taxonomy. Freeman, San Francisco.

Tamura K, Nei M, Kumar S, 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proc. Nat. Aca. Sci.*, **101**: 11030-11035.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S, 2013. MEGA6: Molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evo.,* **30**: 2725-2729.

Wang HK, Zhang TY, Zhang M, 2001. Application of sequencing of 5.8S rDNA, ITS1 and ITS2 on identification and classification of *Alternaria* at species level. *Mycosystema,* **20**: 168-173.

White TJ, Bruns T, Lee SJWT, Taylor JL, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a guide to methods and application.*, **18**: 315-322.

Woudenberg JH, Groenewald JZM, Binder, Crous PW, 2013. *Alternaria* redefined. *Stud. Mycol.,* **75**: 171-212.

Woudenberg, JH, Seidl MF, Groenewald, JZ, de Vries M, Stielow JB, Thomma BPHJ, Crous PW, 2015. *Alternaria* section *Alternaria*: Species, formae speciales or pathotypes? *Stud. Mycol*., **82**: 1-21.

.