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MORPHO-MOLECULAR IDENTIFICATION OF *CERCOSPORA BRACHIATA* CAUSING LEAF SPOT ON *ACHYRANTHES ASPERA* IN INDIA

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ABSTRACT

Cercospora leaf spot disease of *Achyranthes aspera*, a medicinally important plant, caused by *Cercospora brachiata*, is reported for the first time. The identification of the pathogen was confirmed through a combination of morphological and molecular analyses. Koch's postulates were successfully fulfilled with this isolated fungus obtained from Kushmi Forest, Gorakhpur, U.P., India.

Key words : Fungal pathogen, New host, Amaranthaceae, First report.

Introduction

Achyranthes aspera L is a herbaceous species belongs to the family Amaranthaceae. It is now found globally as a weed in tropical and subtropical regions, affecting more than 50 crops (Francischini *et al*., 2014). *A. aspera* is highly adaptable to various environmental conditions and produces a large number of seeds. *Cercospora* is a notable genus within the *Mycosphaerellaceae* (*Mycosphaerellales*) and members of this genus are plant pathogens typically causing leaf spot diseases. This genus is characterized by septate, colourless conidia and coloured conidiophores with thickened and darkened loci and hilum (Crous and Braun, 2003). During a survey conducted in August 2022, fungal infected leaves of *A. aspera* were collected from Kushmi Forest, Uttar Pradesh, India. After comparing the collected fungus with all other species reported on related hosts, we identified the collected fungal sample as *Cercospora brachiata* (Chupp, 1954; Crous and Braun, 2003). This is the first report of *C. brachiata* on medicinally important plant *A. Aspera*, which is used in the treatment of cough, asthma, bronchitis, renal complications (stone) and skin diseases (Ndhlala *et al*., 2015).

Materials and Methods

Infected leaf samples from different parts of Kushmi Forest, Uttar Pradesh, India were placed in separate polythene bags and transported to the laboratory. Surface scrapings and free-hand cut sections were prepared from infected portions, mounted in lactophenol cotton blue and observed under microscope to morph-taxonomic determination as described by Verma *et al.* (2021a, b, c; 2023), Gargee *et al.* (2022), Sanjay *et al.* (2022; 2023a, b). Pure culture was obtained through single-spore isolation technique. DNA was extracted from culture employing modified CTAB method (Conlon *et al*., 2022). Primers EF1-728F/EF1-986R and ACT-512F/ACT-783R were used to amplify alpha 1-elongation factor (TEF-1α) and actin gene (act) (Carbone and Kohn, 1999). The purified PCR product was sequenced and deposited in GenBank. Megablast analysis of TEF-1α and act showed 100% similarity (MK118086) and 99% similarity (MK118087) with *C. brachiata*, respectively. Steps for phylogenetic tree construction were followed as described by Sanjay *et al.* (2022, 2023a, b).

Results and Discussion

Leaves of *Achyranthes aspera* showing amphigenous, circular to irregular infection spots were collected from the Kushmi Forest, U.P., India. On host plant, pathogen colonies were amphiphyllous; mycelium internal; stromata present; conidiophores found in fascicle, straight to flexuous, cylindrical to slightly attenuated

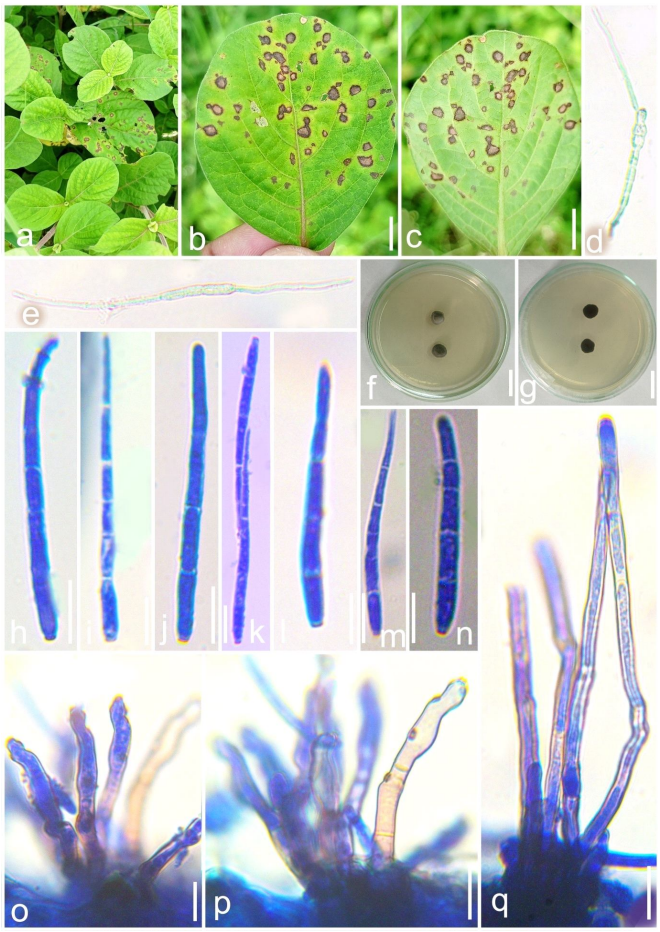

Fig. 1 : *Cercospora brachiata*. **a** Host plant in natural habitat. **b** Symptoms on upper surface of leaf. **c** Symptoms on lower surface of leaf. **d**, **e** Germination conidia on PDA. **f** Top view of culure on PDA, **g** Reverse view of culture on PDA. **h**–**n** Conidia. **o**–**q** Fascicles of conidiophores with stromata. Scale bars: d, c, f, g = 10 mm, h–q = 20 µm.

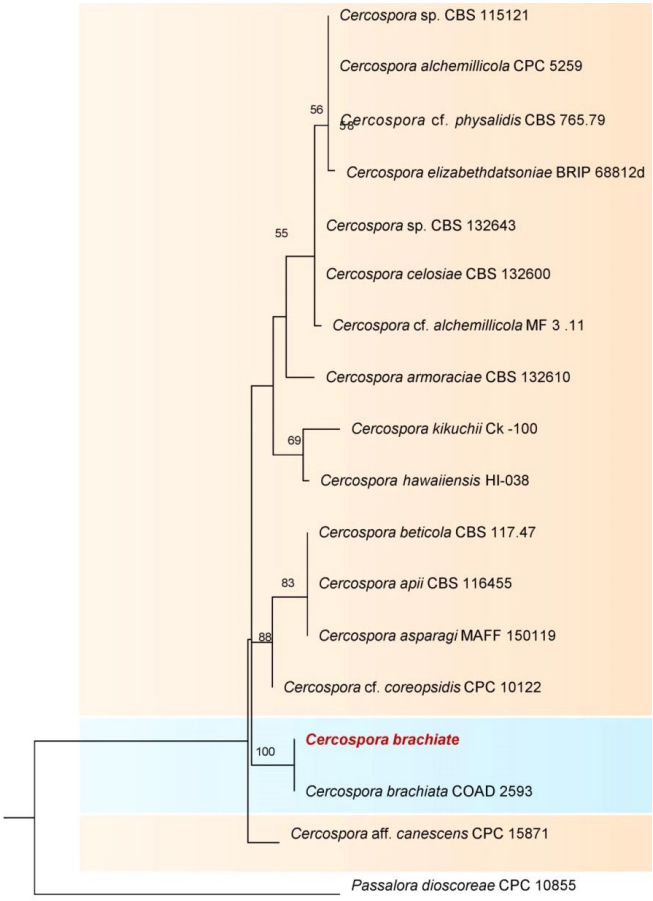

Fig. 2 : Phylogram generated from maximum likelihood analysis based on combined *act-tef-1α* sequence data. 18 strains are included in the combined sequence analysis, which comprise 403 characters with gaps. *Passalora dioscoreae* (CPC 10855) was used as the outgroup taxon. The best scoring *RAxML* tree with a final likelihood value of –1054.753342 is presented. Estimated base frequencies were as follows; A = 0.221222, C = 0.318835, G = 0.246432, T = 0.213511; gamma distribution shape parameter α =0.282283. Bootstrap support values for ML equal to or greater than 50% are given above the nodes. Newly generated sequence is in red.

towards a truncate tip, unbranched, geniculate, light brown, thin-walled, smooth, 1-7-septate, 40-126 × 4.5-5 µm; conidiogenous cells integrated, terminal to intercalary, proliferating sympodially, loci thickened and darkened; conidia dry, simple, solitary, smooth, thin-walled, straight to curved, fusiform, apex acute to subacute, obconically truncate to truncate at the base, 3-8-septate, hyaline, 35-145 × 3-4 µm, hilum thickened and darkened. On the basis of morphological features, it was found similar to *Cercospora brachiata* Ellis & Everh., as described in Chupp (1954).

In culture: Slow-growing (7–8 mm diam. after 14 days in PDA at 25±5^{0}C), aerial mycelium sparse, Based on combined *act-tef-1α* sequence data, the clustering of our isolate with *Cercospora brachiata* (COAD 2593) with high statistical support indicates that isolate is indeed *C. brachiata*. This kind of genetic analysis is a reliable method for species identification, especially when combined with robust statistical support.

Pathogenicity test was performed by putting droplets on leaves of healthy plants with aqueous suspension of spore (10^6 conidia /ml). Plants were covered with sterile transparent plastic bags for 10 days. After 10 days of inoculation, leaf spots similar to that previously described were observed. Koch's postulates were confirmed by the re-isolation of same pathogen from inoculated healthy

leaves after 10 days of inoculation. This is the first report of *C. brachiata* on medicinally important plant *A. Aspera*.

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