African Journal of Microbiology Research Vol. 6(3), pp. 633-636, 23 January, 2012
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR11.1440
ISSN 1996-0808 ©2012 Academic Journals

Full Length Research Paper

First report of *Alternaria astragali* as a causative agent of leaf spot disease in *Polygonum multiflorum* Thunb.

Guoru Xiong[1#], Gengfeng Zhao[2#], Li Li[2] and Shuzhen Zhang[1]*

[1]Institue of Tropical Biotechnology, Chinese Academy of Tropical Agricultural Sciences, HaiKou 571101, China.
[2]Department of Biochemistry, Hainan Medical College, HaiKou 571101, China.

Accepted 10 January, 2012

Leaf necrosis was observed on leaves of *Polygonum multiflorum* Thunb. in Kunming, China, from 2008 to 2009. The causal fungus was isolated and pure cultured from the leaf spot lesions on prostate-specific antigen (PSA). Koch's postulates were fulfilled by inoculating leaves *in vitro* in a moist chamber and *in vivo* in field. Morphological and internal transcribed spacer (ITS) region sequence analysis (FJ379591) revealed that the fungal strain shared 99% nucleotide identity with *Alternaria astragali* (EF110523). This is the first report of *A. astragali* causing leaf spot on *P. multiflorum* Thunb. in China.

Key words: *Alternaria astragali*, identification, internal transcribed spacer (ITS) analysis, leaf spot and *Polygonum multiflorum* Thunb.

INTRODUCTION

Polygonum multiflorum Thunb (the Chinese name called He-shou-wu) is well known as one of the most important and widely used Chinese herbal tonics (Duke and Ayensu, 1985). He-Shou-Wu is widely grown in Yunnan, Henan, Hubei, Guangxi, Guangdong, Guizhou and Sichuan Provinces of China. However, in recent years the production of *P. multiflorum* has been severely hindered by different diseases, especially fungal borne leaf spot disease in recent years (Sang et al., 2007; Lu and Lu., 2008).

This disease occurs mainly on mature or immature leaves, and forms early brown spot, but later enlarge to round, nearly round or irregular lesions which contain concentric brown circles, with a grey center (Figure 1). When drying, the centre of lesion is fragile and wears through. Therefore, during 2008 to 2009, diseased samples from several production areas were collected, and the causal organism was isolated and tested for its pathogenicity. The internal transcribed spacer (ITS) was also sequenced to identify its classification.

MATERIALS AND METHODS

Pathogenicity tests

Leaves of He-shou-wu with grey leaf spot lesions were carefully selected for the isolation of the fungus. The causal fungus was isolated from the lesion and cultured on potato dextrose agar (PDA) culture medium. Pathogenicity tests were conducted by inoculating healthy leaves with a conidial suspension (1×10^6 conidia mL^{-1}). For each test, four detached leaves were inoculated with the conidial suspension and placed in a moist chamber at 25°C for 24 h. Control leaves were sprayed with distilled water. In the field, leaves of plants belonging to the same age group were tagged, inoculated with the fungal conidial suspension, and sealed with a preservative film to keep them moist. The plants were observed for symptom development after three days.

PCR amplification

From extracted genomic deoxyribonucleic acid (DNA), the ITS region 1 and 2 including 5.8S of nuclear rDNA were amplified with ITS1 and ITS4 primers (ITS1:5'- TCCGTAGGTGAACCTGCGG -3', ITS4: 5'- TCCTCCGCTTATTGATATGC -3'). Polymerase chain reaction (PCR) system (25 µl):10 × buffer (plus Mg2+) 2.5 µl,

*Corresponding author. E-mail: zhangsz111 @163.com. Tel: +86-898-66987509. Fax: +86-898-66988337.

# Authors contributed equally to this work.

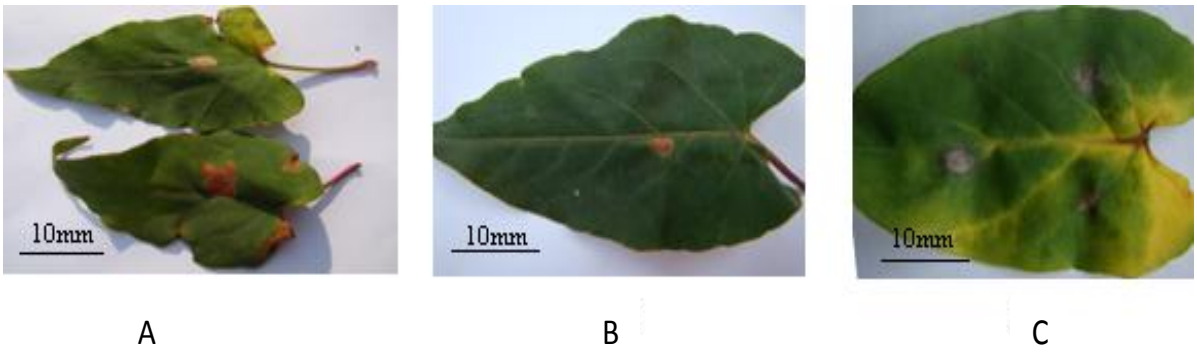

Figure 1. Disease symptom (A: Necrotic symptom of naturally infected *P. multiflorum thunb* with *A. astragali*; B: Necrotic symptom of *P. multiflorum thunb* inoculated by *A.astragali* in vivo at field; C: Necrotic symptom of *P. multiflorum thunb* inoculated by *A. astragali* in vitro at room).

dNTP (each 2.5 mmol/L) 2 µl, ITS1, ITS4 (5 µmo1) each 2 µl, exTaq DNA polymerase (5u / µL) 0.25 µL, extracted genomic DNA: 1 µL, deionized water: 15.25 µL. Each PCR reaction was conducted with 30 thermal cycles according to following conditions: 1 min at 95°C for denaturation; 1 min at 52°C for primer annealing; 1 min at 72°C for extension; 10 min at 72°C for terminal extension. Amplified PCR products were detected on 1.2% agarose gel through electrophoresis (0.04 M Tris–acetate, 0.001 M EDTA) pH 8.0 buffer.

Cloning and sequencing of ITS PCR products

PCR products obtained from selected isolates were separated by electrophoresis on 1.2% agarose gels, excised, and purified using the QIAEX II agarose gel extraction protocol (Qiagen GmbH, Hilden, Germany). Purified dsDNA fragments were ligated into a pGEM-T vector (Promega GmbH, Hilden, Germany) and subsequently transferred into *Escherichia coli* DH5α cells. Nucleotide sequences of the cloned PCR products were determined by a commercial company (SHENGGONG, SHANGHAI, CHINA). Sequencing reactions were primed on both strands using either the T3 or Sp6 promoter sequences of the pUCm-T vector.

RESULTS

The disease under natural infection occurred mainly on mature or immature leaves. At an early stage, it forms only brown spots, but later enlarges to round, nearly round or irregular lesions which contain concentric brown circles, with a grey center (Figure 1A). Upon drying, the centre of the lesion becomes fragile and wears through. With ample moisture, black mildew of fungus, which are conidia and conidiophores appear on the lesions.

Pathogenicity

The pathogen produced a grey, felt-like, with slightly irregular edge colonies on PDA culture medium. The hypha produced simple, dark-colored and erect conidiophores that bore single or branched chains of conidia. The conidial bodies were ovoid or predominantly long-ellipsoid, 20 to 45 × 3 to 4.5 µm, with 4 to 6 transverse septa and 1 to 5 longitudinal septa. The peak (base of conidia) was columnar, light brown and septate and measured 4.5 to 30.0 × 3.5 to 4.5 µm in size (Figure 2). Koch's postulates were fulfilled by inoculating healthy leaves of the tree with the conidial suspension isolated from the naturally infected plants. Within 7 to 10 days, symptoms similar to those observed on naturally infected leaves appeared (Figures 1B and C). Control leaves sprayed with distilled water did not develop any symptoms. The re-isolated causal fungus was identical to that from the leaves on which lesions developed after inoculation.

Sequence analysis of the ribosomal DNA spacer sequences (ITS)

The 600 bps nucleotide sequences of the ITS region were obtained and submitted to national center for biotechnology information (NCBI) Genbank (Accession number FJ379591). Homology was searched by BLAST software for HSW-1, and it is showed as 99% sequence homology with *Alternaria astragali* (EF110523) (Wangeline and Reeves, 2007). Phylogenetic tree was constructed with selected strains using DNAMAN software (Figure 3). Phylogenic analysis showed that the isolates tested (FJ379591) and the *Alternaria* strains were clustered into the same large clade, showing close evolutionary relationship between HSW-1, *A. tenuis* (EU520059), *Alternaria abutilonis* (AF314578) and *A. astragali* (EF110523).

DISCUSSION

On the basis of morphological and molecular characteristics, the causal fungus was thus identified as a

Figure 2. The conidia of *Alternaria astragali.*

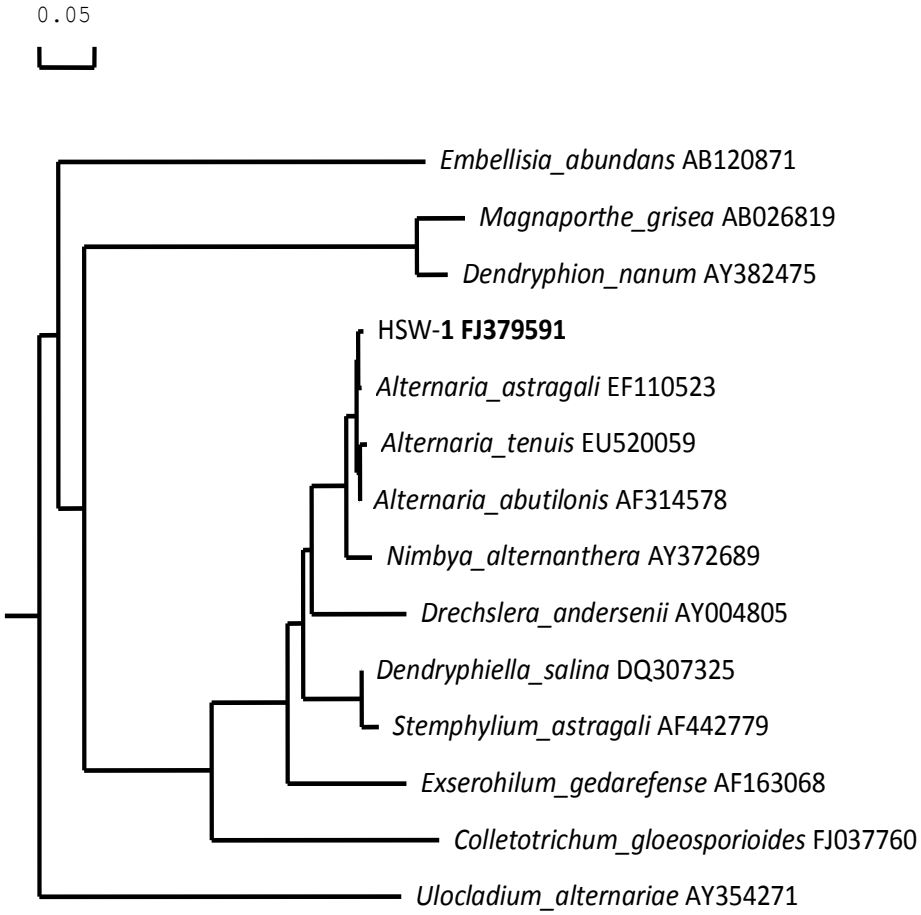

Figure 3. Phylogenetic tree based on the 18S rDNA sequence of HSW-1 with its relating fungi.

new strain of *A. astragali*. It was, therefore, concluded that the causal agent of the leaf spot disease in He-shou-wu plant is a new strain of *A. astragali*. As we known, this is the first report of *A. astragali* on *P. multiflorum* Thunb. in china. This information would be useful for designing methods to control the disease. However, further studies on the epidemiology, disease cycle, prevention, and control strategies of the pathogen are needed.

ACKNOWLEDGMENT

This study was supported by the Modern Agriculture Industry Technology System Construction of Special Funds (nycytx-24).

REFERENCES

Duke JA, Ayensu ES (1985). Medicinal Plants of China. Reference Publications, Inc.

Lu LJ, Lu S (2008). Technology of controlling diseases and pests on *Polygonum multiflorum thunb* . Plant Doctor, 21(2): 21-22.

Sang WJ, Li XX, Lian QX, Li XY, Yang H, Cai LY (2007). Studies on biological characteristic of black rot in Dendrobium nobile of Chishui. J. Yunnan Univ. Nat. Sci., 29(1): 90-93.

Wangeline AL, Reeves FB Jr (2007). Two new Alternaria species from selenium-rich habitats in the Rocky Mountain Front Range. Mycotaxon, 99: 83-89.