

# First Report of Marginal Scorch Infecting Indigowoad Root in China

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## Abstract

During 2015, marginal scorch symptoms were detected in the production base of indigowoad root Hubei, China. On the basis of morphological features and 18S rDNA sequences, the pathogen was identified as *Cladosporium* sp. Koch's postulates were fulfilled by pathogenicity tests on potted indigowoad root seedlings. To our knowledge, this report is the first of marginal scorch on indigowoad root caused by *Cladosporium* sp. We propose the name "marginal scorch" for the new disease.

## Keywords

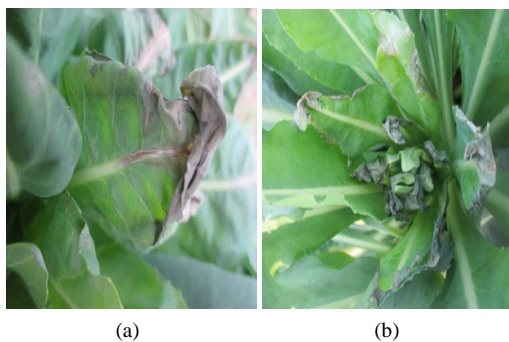
Indigowoad Root, Marginal Scorch, *Cladosporium* sp.

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Indigowoad root (*Isatis tinctoria*) is one of the most well-known approved prescription remedies and is frequently used as an anti-leukemia, antipyretic, anti-inflammatory and anti-virus agent [1]. In addition, a compound from indigowoad root granules has been accredited as antiviral agent against influenza virus [2]. Moreover, the fresh leaves are used as a vegetable. As there is more demand for healthy and nutritional life style, the medicinal role of indigowoad root will get more attention in future. As an important medical vegetable, the planting area of indigowoad root has significantly increased. However, the symptom of the marginal scorch for indigowoad root was observed in the Wuhan City and Shennongjia Forestry District, Hubei Province, China (Figure 1(a)). First the margin of leaf became yellow, then gradually crisping and brown. The incidence of symptoms was almost 100%, seriously affecting the commercial quality of the leaves from indigowoad root. Therefore, the pathogens were isolated by tissue segment method on potato dextrose agar medium [3]. A suspension containing  $10^5$  conidiophores per ml collected from 7-day-old colonies grown on PDA was sprayed on the foliage of indigowoad root. The control plants were inoculated with sterile water. After inoculation, the plants

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**Figure 1.** The symptom of marginal scorch infecting indigowoad root. (a) Symptoms in field; (b) symptoms after inoculation.

were placed at 25°C and 80% humidity. The first lesions appeared after 7 day (**Figure 1(b)**). Koch's postulates were fulfilled by consistently reisolating pathogens from inoculated plants, whereas control plants remained healthy. The genomic sequence of 18S rDNA was studied. The DNAs of mycelia were extracted with CTAB method [4]. The sequences of primers were designed as follows: 18S-1,5'-GTAGTCATATGCTTGTCTC-3'; 18S-2, 5'-TCCGCAGG TTCACCTACGGA-3'. The PCR reaction conditions were as follows: The settings for the thermal profile included an initial denaturing at 94°C for 2 min, followed by 25 cycles of amplification (94°C for 30 s; 45°C for 30 s; and 72°C for 2 min) and a finally extension at 72°C for 5 min. The PCR products were detected by 1% agarose gel electrophoresis. A band was detected around 1700 bp. The amplification products were ligated, transformed and sequenced. The results were aligned with the sequences in GenBank. The 18S rDNA sequence of the representative pathogen isolated from the fresh leaves of the indigowoad root was deposited in GenBank (accession no. KU512834). The basic local alignment search tool (BLAST) was used to indicate functional and evolutionary relationships between sequences, identifying members of gene families. BLAST search of this nucleotide sequence illustrated 99% identity with 18S rDNA sequences of several *Cladosporium* sp. isolates available in GenBank. So the species was identified as *Cladosporium* sp. This is the first reported *Cladosporium* sp. infection in fresh leaves of the indigowoad root in China, and we believe that this information will be useful for studying *Cladosporium* sp. infection in other vegetables. At the same time, our results indicate the need for the revision of indigowoad root management.

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