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Short Communication

Marginal scorch caused by *Alternaria alternata* on Purple-Caitai (*Brassia campestris* L. ssp. c*hinensis* L.var. *utilis* Tsen et Lee) in China

Qijun Nie, Zhongjiu Jiao, Fengjuan Zhu, Zhenbiao Jiao, Xiaohui Deng, Zhengming Qiu and Jinping Wu*

Institute Industrial Crops, Hubei Academy of Agricultural Sciences, Wuhan 430064, China.

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A new disease was detected in Purple-Caitai (*Brassia campestris* L. ssp. c*hinensis* L.var. *utilis* Tsen et Lee) on the high mountain in Hubei, China. On the basis of morphological and cultural features, the pathogen was identified as *Alternaria alternata*. The internal transcribed spacer (ITS)-rDNA sequence of a representative *Alternaria* isolate from Purple-Caitai showed 99% identity with other ITS sequences of different *Alternaria alternata* isolates available in GenBank, thus confirming the morpho-cultural identification. Koch′s postulates were fulfilled by pathogenicity tests on potted Purple-Caitai seedlings. To our knowledge, this report is the first of marginal scorch on Purple-Caitai caused by *Alternaria alternata*.

Key words: Purple-Caitai, marginal scorch, *Alternaria alternata*

INTRODUCTION

Purple-Caitai (*Brassia campestris* L. ssp. *chinensis* L.var. *utilis* Tsen et Lee. 2n=2x=20) is a variant of *Brassica* that originated from the central part of the Yangtze River Region (Xiao, 2008). Due to its good taste, crisp refreshing and rich nutrition, Purple-Caitai became people's favorite vegetable in China. In early summer of 2010 to 2011, when the temperature was 20 to 25°C, a novel disease, designated marginal scorch, was found in Purple-Caitai in the Changyang Tujia Autonomous County of Yichang City and Enshi Tujia Autonomous Prefecture of Lichuan City, Hubei Province, China. Initially, the margin of leaf becomes yellow, then crispy and brown. At the final stage of the disease, infected plants was withered and yellow although the roots appeared to be healthy. The downy mildew (Farinhó et al., 2007), soft rot disease (Shim et al., 2011), clubroot (Cho et al., 2012) and so on had been reported in *Brassica*, but the symptoms of the disease had not been reported. The present study aimed at identifying the causal agent of marginal scorch.

*Corresponding author. E-mail: wjp9188@yahoo.com.cn. Tel: 86 27 87380187. Fax: 86 27 87380187.

MATERIALS AND METHODS

Isolation of pathogen causing marginal scorch

Margin scorch was observed on leaves of Purple-Caitai (Figure 1a). The pathogens were isolated by tissue segment method (Fang, 1996) on potato dextrose agar (PDA) medium. Infected Purple-Caitai leaves were cut into small pieces of 1.0 to 1.5 cm; surface sterilized with 0.1% mercuric chloride for 1 min, and washed in sterile distilled water thrice and blotted dry with sterilized filter paper. Then, the leaf pieces were placed in Petri plates containing PDA. The plates were incubated at 28 ± 2°C for seven days and observed for fungal growth.

Phenotypic characterization and pathogenicity tests

The morphological characters of pathogen were observed by light microscopy (Nikon eclipse 90i) and it was incubated for seven days at 28°C on PDA. At the same time, we made use of the Koch's postulates to prove that the fungi were the cause of the Purple-Caitai disease. A suspension containing 10^5 conidiophores per ml collected from seven-day-old colonies grown on PDA was sprayed on the foliage of 30 Purple-Caitai seedlings. The same numbers of control plants were inoculated with sterile water. After inoculation, the plants were kept in a growth chamber at 25 ± 2°C. Marginal scorch symptoms were observed every 12 h. Each treatment was repeated three times.

Figure 1. Natural symptoms of Purple-Caitai (*Brassia campestris* L. ssp. *Chinensis* L.var. *utilis* Tsen et Lee) margin scorch and the symp-toms of Purple-Caitai at 7days post-inoculation. **a.** Natural symptom. **b.** Post-inoculation symptoms.

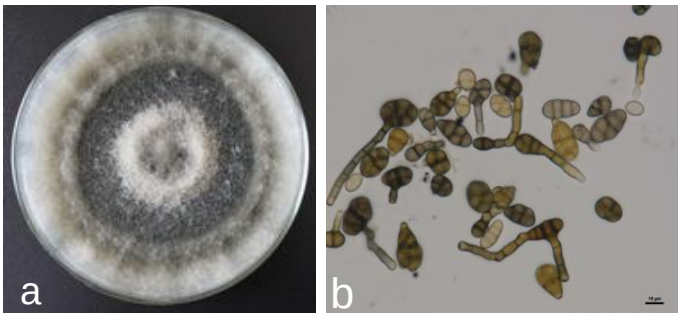

Figure 2. The morphology of the pathogen for growing on PDA agar for 7 days at 28°C. **a.** Colony of *A. alternata* on PDA. **b.** Conidial mass of *A. alternata* (scale bar 10 µm).

Phylogenic characterization

Genomic DNA was extracted from a suspension culture of *A. alternata* by the cetyltrimethyl ammonium bromide (CTAB) (Knapp and Chandlee, 1996). The identification was further confirmed using the rDNA and ITS primer (Jung et al., 2002). Specific primers for 18S rDNA, were ITS-F (5'-GTCCTAACAAGGTTTCCGTA-3'; AJ297952) and ITS-R (5'-TTCTCCGCTTATTGATATGC-3'; AJ297953). The PCR amplification conditions were as follows: one denaturation step (3min at 95°C), 35 cycles of amplification (30s at 95°C, 60s at 57°C, 2 min at 72°C), and a final elogation step of 10 min at 72°C. The specific bands were selected, cloned, sequenced and the sequences obtained were ''blasted'' in GenBank.

RESULTS AND DISCUSSION

Phenotypic characterization and pathogenicity tests

The fungus produced abundant, branched, septate, brownish mycelia (Figure 2a). Conidia were light brown to dark brown and brick shape with three to five transverse septa (Figure 2b). While the morphological characteristics were influenced by nutrient medium and temperature, humidity, light, pH and so on, it was very difficult to accurately identify the species.

At the same time, the first lesions appeared after seven days (Figure 1b). Koch's postulates (Koch, 1893) were fulfilled by consistently reisolating *A. alternata* from inoculated plants. The repeated experiments confirmed the same results.

Phylogenic characterization

ITS has been widely applied to different kinds of fungi of the genus within or similar genera in phylogenetic studies (Chen and Zheng, 2007; Yuan et al., 1995). Sequence analysis of the ITS substantially reflect the genus, species and strain differences between base-pairs. The ITS-rDNA sequence of the representative pathogen isolated from Purple-Caitai was deposited in GenBank (accession no. JQ885954). The basic local alignment search tool (BLAST) can be used to infer functional and evolutionary relationships between sequences as well as help identify members of gene families. BLAST search of this nucleotide sequence showed 99% identity with ITS sequences of several *A. alternata* isolates available in GenBank (accession no. EF471931). On the basis of morphological and molecular features, the species was identified as *A. alternata*.

The present study reveals the *A. alternata* infection Purple-Caitai in hubei Province, China. This is the first *A. alternata* survey in Purple-Caitai in China, and we believe that this information will be significant for researching the *A. alternata* infection in Purple-Caitai and other vegetable. At the same time, the finding shows the need for the revision of Purple-Caitai management.

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