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Short communication

Occurrence of powdery mildew caused by *Golovinomyces orontii* on *Lactuca sativa* var. *ramosa* (lettuce) in China

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ABSTRACT

Lettuce (*Lactuca sativa* L. var. *ramosa* Hort.) is one of the most popular leafy vegetables in China, which contains abundant vitamin C and moderate iron. In August and October 2017, two different lettuce cultivars, heading type with green leaves (iceberg lettuce) and looseleaf type with red leaves, infected by powdery mildews were collected in Jilin Agricultural University (43.81°N; 125.41°E), Changchun, China. The disease incidence was approximately 60–70%. This disease initially formed small, amphigenous and indistinct white colonies, finally covering whole leaf surfaces, which caused leaf yellowing and withering and severely influences on yield and quality. Conidia were formed in short chains (catenescent) without fibrosin bodies. Chasmothecia were not observed on the collected samples. Based on the morphological characteristics of the fungus on lettuces, internal transcribed spacer (ITS) and 28S rDNA sequences and pathogenicity, the pathogen was identified as *Golovinomyces orontii*. It is the first report of *G. orontii* infections on lettuce in China. The information may be helpful for disease monitoring to lettuce growers and breeders.

1. Introduction

Lettuce (*Lactuca sativa* L. var. *ramosa* Hort., Asteraceae) is a leafy green vegetable often used for salads, soups, wraps or sandwiches. It is cultivated both in open fields and in greenhouses in China. Iceberg lettuce belonging to heading lettuce type is also known as crisphead or cabbage lettuce (De Vries, 1997). Lettuce powdery mildew significantly influences the quality of lettuces, and is often considered as a minor or secondary disease (Blancard et al., 2006). The epidemics of lettuce powdery mildew have been observed in lettuce crops in drier, warmer areas of the USA (e.g. Arizona and California), including the warm southern part of the Salinas Valley (Ryder, 1999). Recently, the climate change may be one of the factors influencing disease epidemiology (Garrett et al., 2006) and may increase the impact of powdery mildews on lettuce.

Powdery mildews were previously reported as *Erysiphe polygoni*, *Sphaerotheca fuliginea* (Tai, 1979), *E. communis*, *E. cichoracearum* (Amano, 1986), *Oidium* sp. (Zhuang, 2005), *Podosphaera fusca* (Shin et al., 2006), *Golovinomyces cichoracearum* (Braun and Cook, 2012; Lebeda and Mieslerová, 2011) and *G. orontii* (Cho et al., 2016) on *Lactuca sativa* L. There is no record of *G. orontii* on genus *Lactuca* in Braun and Cook (2012). The main aim of this article is to precisely identify the species of powdery mildews on lettuces collected in Changchun, China, based on morphological characteristics, ITS and partial 28S rDNA sequences and pathogenicity tests of the fungus.

2. Materials and methods

Two different lettuce cultivar varieties (iceberg lettuce is heading type with green leaves and red leaf lettuce is looseleaf type respectively) infected by powdery mildews were collected from two vegetable gardens at least 500 m apart from one another in August and October 2017 in Jilin Agricultural University, Changchun, China. The infected plant samples were dried and used as herbarium specimens deposited in the Herbarium of Mycology of Jilin Agricultural University under accession no. HMJAU91769 and HMJAU91770.

Conidia from fresh collections were examined in water for the presence or absence of fibrosin bodies using light microscopy (ZEISS Axio Scope. A1, Germany). The dimensions of conidia and conidiophores (at least 30) were measured in distilled water. Germination of conidia were examined following the method of Hirata (1942). The inner surfaces of onion scales covered with conidia were floated on

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distilled water in a Petri dish, and were placed on the laboratory bench at room temperature around 20–25 °C for about 12 h, and then microscopically examined.

Total fungal DNA was extracted from the mycelia and conidia collected from the infected leaves by the Chelex-100 method (Walsh et al., 1991; Hirata and Takamatsu, 1996). The complete ITS region including 5.8S rDNA and partial 28S rDNA sequence of the pathogen were amplified by PCR with primer pairs ITS4/ITS5 and LSU1/LSU2, respectively, and then sequenced. The reaction components were 2 μL of total genomic DNA, 2.5 μL 10 × PCR buffer (Mg^{2+} plus) (TaKaRa, Japan), 2 μL dNTP mixture (10 mM total, 2.5 mM each), 1 μL each primer (20 ng/μL), 0.1 μL Taq polymerase (TaKaRa, Japan) (5 U/μL) and sterile ddH_2O up to a final volume of 25 μL. PCR reactions were conducted under the following thermal cycling conditions: an initial denaturation step of 3 min at 95 °C, 35 cycles of 30 s at 95 °C, followed by 30 s at 56 °C for annealing, and 30 s at 72 °C for extension, and a final extension for 7 min at 72 °C. A negative control that just lacked the template DNA was included in each set of reactions. PCR products were subjected to electrophoresis in a 1.2% agarose gel in 0.5 × TBE buffer. The assembled sequences gained in this study were deposited in DNA databases (GenBank). The ITS and 28S rDNA sequences were compared with entries in the National Center for Biotechnology Information database (NCBI, http://www.ncbi.nlm.nih.gov/Blast.cgi) using the BLASTn program hosted at Nucleotide BLAST.

The pathogenicity test was conducted by gently dusting a diseased leaf onto six healthy leaves of lettuce, which was repeated twice. Three non-inoculated leaves served as controls. Leaves were maintained in an incubator at 24–26 °C and relative air humidity of 60%.

3. Results and discussion

During autumn 2017, severe powdery mildew infections with approximately 60–70% incidence were found on lettuces cultivated in vegetable gardens in Jilin Agricultural University (43.81°N, 125.41°E), Changchun, China. The disease initially formed small, amphigenous and indistinct white colonies on the leaves (Fig. 1 A, C). As the disease progressed, white mycelia practically covered the whole of the leaves, and caused a dusty white appearance of the plants (Fig. 1 B, D).

The powdery mildew formed whitish colonies over the surface of lettuce leaves. Mycelia were amphigenous with nipple-shaped or slightly lobed hyphal appressoria (Fig. 2 A, B). Conidiophores arose laterally or from the upper surface of hyphal mother cells, and were 84–175 × 9.2–12.5 μm. Foot-cells were erect or curved at the base, (35.8) 41.1–97.8 × 9.0–13.1 μm, followed by 1–3 short cells (Fig. 2 C, D). Conidia were formed in short chains (catenescent), hyaline, elliptical to ovoid, 24.3–49.4 × 13.0–20.5 μm with a length/width ratio varying from 1.3 to 3.6 and lacked distinct fibrosin bodies (Fig. 2 E). Germ tubes were short to very long, and produced in perihilar or apical position (Fig. 2 F, G). The morphological characteristics were consistent with *Golovinomyces cichoracearum* (Braun and Cook, 2012) and *G. orontii* (Cho et al., 2016), respectively. Chasmothecia were not observed on the collected samples.

Two 618-bp ITS sequences and two 636-bp partial 28S rDNA sequences were obtained from the two lettuce powdery mildew samples, and were deposited in GenBank (accession No. MG148337 and MG263993 for ITS, and MG148338 and MG263994 for partial 28S rDNA). BLASTn analysis of ITS fragments (showed 100% identity with the ITS sequence of *G. orontii* (previously named *G. cichoracearum*) isolates on *Lactuca sativa* (AB769447) and *L. scariola* (AB077688) from Japan and 99.11% identity on *L. serriola* from Korea (KP260660). The partial 28S rDNA sequences also showed 100% identity with the 28S rDNA sequence of *G. orontii* (previously named *G. cichoracearum*) on *L. scariola* from Japan (AB077687).

The pathogenicity tests showed that symptoms developed 5 days after inoculation, whereas the non-inoculated leaves remained symptomless. The fungus from the inoculated leaves was morphologically consistent with that observed from initially diseased plants.

Powdery mildews on *L. sativa*, previously referred to as *E. cichoracearum* and *G. cichoracearum*, respectively, is widespread and known from many countries, such as Australia, the United States, Brazil, Canada, Greece, France, Italy, Romania, South Africa, etc. (Farr and Rossman, 2017). The powdery mildew on *Lactuca* spp. was previously assigned to *G. cichoracearum* [s. lat.] (Braun and Cook, 2012), but is now referred to as *G. orontii* [s. lat.] based on the results of Matsuda and

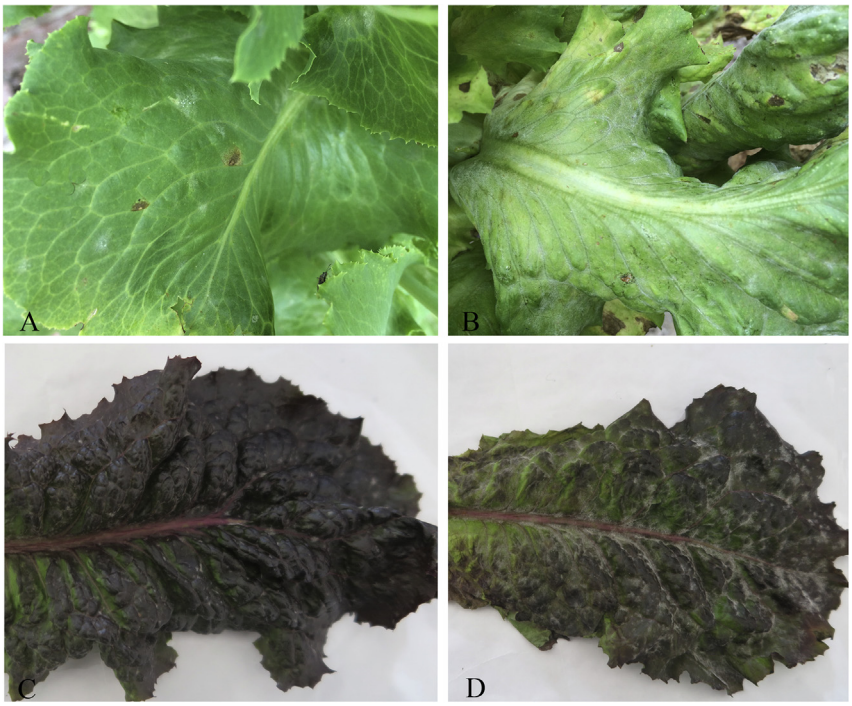

Fig. 1. The symptoms of *Lactuca sativa* var. *ramosa* infected by powdery mildew. A. The initial symptoms on heading lettuce; B. Late symptoms on heading lettuce; C. Initial symptoms on looseleaf lettuce; D. Late symptoms on looseleaf lettuce.