RESEARCH NOTE

Occurrence of Clubroot Caused by *Plasmodiophora brassicae* in Baecheongchae

Wan-Gyu Kim[1*], Sang-Keun Oh[2], Marc Semunyana[2], Man-Jong Han[1], Gyo-Bin Lee[1], and Weon-Dae Cho[1]

[1]Global Agro-Consulting Corporation, Hwaseong 18330, Korea

[2]Department of Applied Biology, Chungnam National University, Daejeon 34134, Korea.

*Corresponding author: wgkim5121@naver.com

ABSTRACT

Clubroot symptoms were frequently observed on the roots of Baecheongchae plants grown in vinyl greenhouses of a farmer located in Yangpyeong area of Korea during a disease survey in June 2019. The incidence of diseased Baecheongchae plants ranged from 30 to 90% in the vinyl greenhouses investigated. Many resting spores were found in the tissue of root galls collected. The resting spores were hyaline and spherical and measured 2.5–4.2 μm in diameter. Three inoculum suspensions of resting spores prepared from the root galls were inoculated to the roots of healthy Baecheongchae plants. All the inoculum suspensions caused clubroot symptoms to appear on the roots of the inoculated Baecheongchae plants. The symptoms on the roots induced by artificial inoculation were similar to those observed in the plants of the vinyl greenhouses during the disease survey. Resting spores of the pathogen were recovered from the root galls of the inoculated plants. Three root gall isolates obtained from the inoculated plants were used for molecular identification. Comparing the isolates to the *Plasmodiophora brassicae* strains in GenBank, the amplification products demonstrated 100% similarity with the internal transcribed spacer (ITS2) sequences. The clubroot pathogen was identified as *P. brassicae* according to its morphological, pathological, and molecular characteristics. This is the first report of *P. brassicae* causing clubroot in Baecheongchae.

Keywords: Baecheongchae, clubroot, pathogenicity, *Plasmodiophora brassicae*

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Baecheongchae is a new crop that is produced through the hybridization of Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*) and Pak-Choi (*B. rapa* L. ssp. *chinensis*). The crop is produced by Asia Seed Company located in Seoul, Korea, and has recently been grown in many locations. However, information on the origin and characteristics of the crop is not reported. The species of the crop is presumed to be *B. rapa*, but the name of its subspecies is unknown. The crop is cultivated as a popular vegetable in Korea.

Clubroot symptoms were frequently observed on the roots of Baecheongchae plants grown in vinyl greenhouses of a farmer located in Yangpyeong area of Korea during a disease survey in June 2019. The infected plants were retarded in growth, and their lower leaves turned yellow (Figs. 1A and 1B). Severely diseased plants withered and dried up. The symptoms appeared as galls on the roots (Fig. 1C). Four vinyl

greenhouses of Baecheongchae were investigated for disease occurrence. One hundred plants in each vinyl greenhouse were investigated in three replicates. The incidence of diseased Baecheongchae plants ranged from 30 to 90% in the vinyl greenhouses investigated.

Root galls of diseased Baecheongchae plants were collected from the vinyl greenhouses for examination and inoculation experiments. The root galls were sectioned using a scalpel and observed under a light microscope. Many resting spores were found in the tissue of the root galls (Fig. 1D). The resting spores were hyaline and spherical and measured 2.5−4.2 ㎛ in diameter. The morphological features of the isolates were similar to those of *Plasmodiophora brassicae* Woronin described in a previous study [1].

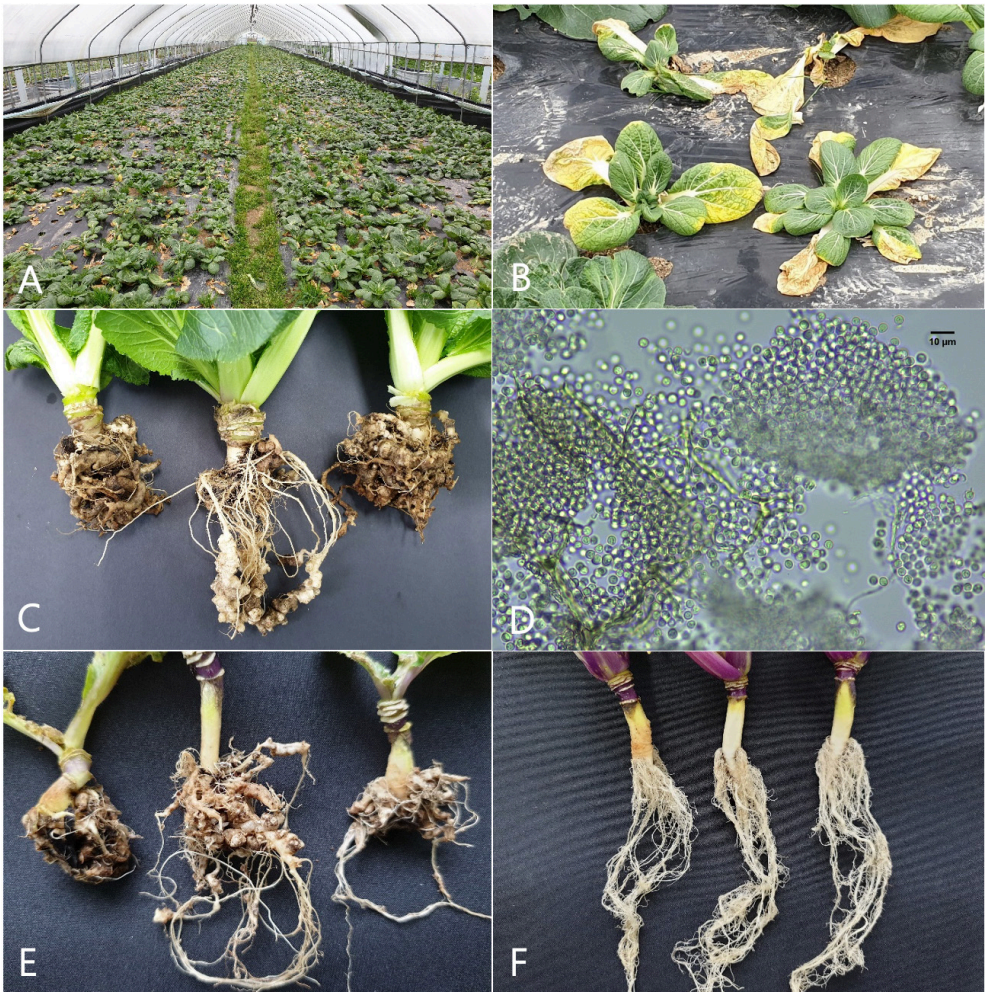

Fig. 1. Clubroot symptoms of Baecheongchae and detection of the pathogen, *Plasmodiophora brassicae*. A and B, aboveground symptoms of clubroot on Baecheongchae plants observed in the vinyl greenhouse; C, galls produced on the roots of the diseased plants; D, resting spores released from a root gall tissue observed under a light microscope; E, root galls induced by artificial inoculation tests with *P. brassicae*. F, non-inoculated plants (control) showing normal growth of roots.

Inoculation tests were conducted following the procedure of a previous report [2]. Three root galls of Baecheongchae were collected and used for inoculum preparation of the pathogen. An inoculum suspension of resting spores was prepared from each of the root galls collected. The inoculum suspension was adjusted to a concentration of $1-2\times10^7$ spores/mL using a hemocytometer. Three inoculum suspensions were inoculated to the roots of healthy Baecheongchae plants grown in circular plastic pots (height, 9 ㎝; upper diameter, 10 ㎝; lower diameter, 7 ㎝) in the vinyl greenhouse. Twenty milliliters of each inoculum suspension were poured into the roots of 23-day-old Baecheongchae plants. The same quantity of sterile distilled water was used as the control. Disease ratings were calculated based on the degree of gall formation on the roots 50 days after inoculation. The inoculation test was performed in five replicates.

All the inoculum suspensions caused clubroot symptoms on the roots of the inoculated Baecheongchae plants (Fig. 1E). The symptoms on the roots induced by artificial inoculation were similar to those observed in the plants of the vinyl greenhouses during the disease survey. No symptom was observed on the roots of the control plants (Fig. 1F). Resting spores of the pathogen were recovered from the root galls of the inoculated plants. Three root gall isolates were obtained from the inoculated plants and used for molecular identification.

To validate the molecular identification of the inoculums, genomic DNA was extracted from the three root gall isolates of Baecheongchae using Cao's method [3]. The internal transcribed spacer (ITS2) rDNA region was amplified with the primers (Pq-forward: 5'-GCAAGACAATGAGCTTTGCTG-3' and Pq-reverse: 5'-TGTGTGTGTCGATCTGCGATT-3') [4]. The protocol for polymerase chain reaction (PCR) amplification was performed as described by Choi et al. [4]. The PCR products were purified (Fig. 2A) and directly sequenced using the same primers. The obtained sequenced data were compared to seven *P. brassicae* strain sequences available in the National Center for Biotechnology Information (NCBI) GenBank database. Comparing our isolate *(P. brassicae* BCH-1) to the *P. brassicae* strains in GenBank, the amplification products demonstrated 100% similarity with the ITS2 sequences (Fig. 2B).

P. brassicae is a soil-borne obligate parasite belonging to a protist. The pathogen causes clubroot in crucifers [5]. It has been reported that the occurrence of clubroot in Chinese cabbage [6] and Pak-Choi [7] is severe in Korea. However, there has been no report on the disease occurrence in Baecheongchae worldwide. In this study, the clubroot pathogen of Baecheongchae was identified as *P. brassicae* according to its morphological, pathological, and molecular characteristics. This is the first report of *P. brassicae* causing clubroot in Baecheongchae.

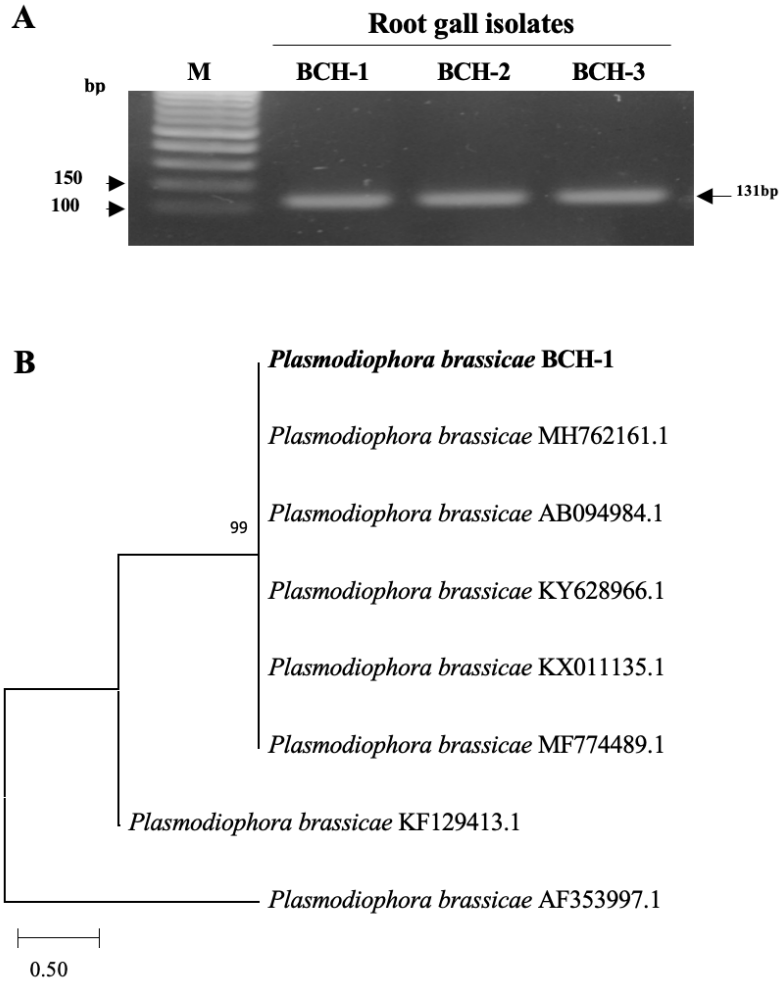

Fig. 2. PCR detection of *Plasmodiophora brassicae* from root gall isolates and phylogenetic analysis of the pathogen, *P. brassicae* BCH-1. A, genomic DNA was extracted from three root gall isolates of Baecheongchae. The ITS2 rDNA region was amplified with the Pq-F/R primers; B, neighbor-joining phylogenetic tree based on a concatenated alignment of the internal transcribed spacer (ITS2) sequence [8]. Numbers on branches indicate bootstrap values (500 replicates). The pathogen strain isolated in this study is in bold.

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