First Report of Phytophthora Leaf Blight and Vine Rot of Kudzu (*Pueraria lobata*) in Korea

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A disease causing leaf blight and vine rot was recognized on kudzu plants (*Pueraria lobata*) in Korea since 1991. A species of *Phytophthora* has been repeatedly isolated from the infected leaves. Identification in species level of the *Phytophthora* sp. remained unsolved. An isolate, KACC 47616 originally collected from Manchon Park in Daegu, has been kept in our laboratory. In 2013, three new isolates, KACC 47617 and KACC 47618 from Yeongyang and KACC 47619 from Gunwi in Gyeongbuk province, were collected and examined to classify up to species level by characterizing morphology, response to temperature and phylogenetic relationship. On the basis of morphological characters such as the nature of hyphal swelling, sporangia and sex organs, absence of chlamydospore production, optimum temperature for mycelial growth, and internal transcribed spacer rDNA and cytochrome oxidase subunit 1 sequence analysis of the pathogen, the causal fungus of kudzu plant was identified as *Phytophthora asiatica*.

**Keywords:** Black locust, *Phytophthora asiatica*, *Phytophthora cinnamomi* var. *robiniae*, *Pueraria lobata*, Robinia pseudo-acacia

The kudzu plant (*Pueraria lobata*) belongs to family Fabaceae, subfamily Faboideae and is a perennial leguminous vine native to Asia, which grows wild on the mountains or roadsides and climbs, coils and trails on other plants, trees and shrubs, and kills them by rapid growth and heavy shading. The plant is, therefore, considered nowadays as a severe noxious mountain weed by foresters. On the other hand, it is a useful plant for medicinal use, and fiber production as well as food being rich in carbohydrate (Wong et al., 2011). In Korea, kudzu plants have been used for production of Korean traditional wall paper and its roots were used for making a drink or tea, but more for oriental medicine to treat diseases such as inner ear dysfunction, fevers, gastrointestinal disorders, skin problems, migraine headaches, lowering cholesterol, and treating chronic alcoholism (Han, 1988; Lee, 1985; Lee, 2004; Yu et al., 2010).

Since 1991, a Phytophthora disease has been identified on those plants. This disease initially produces light brown irregular lesions on leaves, petioles, shoots and vines, later turning to dark brown necrosis lesions and then shrinking and drying, leading to death of the whole vine (Fig. 1A–C) (Rural Development Administration, 2000). The fungus was isolated from the specimens collected from Manchon mountain park in Daegu, Korea. Pathogenicity of the fungus was confirmed by placing the V8 agar culture blocks cut in about 5×5 mm on tender leaves and vines of kudzu plants in agricultural experiment farm on Daegu campus of Kyungpook National University late afternoon on a rainy day in July, and re-isolating the fungus from the lesions formed 4...
to 5 days after inoculation. The pure culture was stored as lab collection. The similar fungus was also isolated from the shoots and leaves of a black locust (Robinia pseudo-acacia) plant growing near the infected kudzu vines (Fig. 1D). In 2002, Ho reported a new taxon of Phytophthora in black locusts and named Phytophthora cinnamomi var. robiniae. Additionally, Rahman et al. (2014) also reported Phtophthora asiatica as the causal fungus of kudzu plants in Japan. However, identification of the species of Phytophthora causing leaf blight and vine rot of kudzu in Korea has remained unsolved. Around that time, in 2013, an effort was made to identify the causal fungus up to species level by collection of more kudzu isolates from different areas of Korea at our laboratory and DNA analysis.

Four isolates of Phytophthora sp., one from Manchon mountain park in Daegu, two from Suha valley, Yeongyang, and one from Seokguram No.2 in Gunwi, were isolated from infected leaves and vines of kudzu plants. All isolates were cultured on water agar (WA) and V8 juice agar (V8). The four isolates were deposited to KACC (Korean Agricultural Culture Collection, Wanju, Korea) and numbered as KACC47616 through KACC47619, respectively. Mycelial blocks were put in sterile distilled water (SDW) to produce many sporangia. Fifty items each of sporangia, antheridia, oogonia, oospores and 20 each of sporangia opening and zoospores were measured for their dimensions.

According to Waterhouse (1963), the morphological characteristics of the kudzu isolates were quite similar with Phytophthora erythroseptica as mentioned by Ho et al. (1983). Therefore, P. erythroseptica, KACC 40712, was received from KACC to compare the effect of temperature on all isolates as the optimum and maximum temperatures of the fungus are already known as 27.5°C and 34°C, respectively (Erwin and Ribeiro, 1996; Ho and Jong, 1989). Potato dextrose agar (PDA) was used to examine the effect of temperature on mycelial growth of kudzu isolates and P. erythroseptica. Mycelial plugs 5 mm in diameter of each isolates were cut from the edge of actively growing mycelia on V8 juice agar by a cork borer, and were placed at the center of PDA plates. Seven different temperature levels (10°C, 15°C, 20°C, 25°C, 30°C, 35°C, 40°C) were set for incubation. Colony diameter was measured daily from 48 hours post seeding until the mycelial growth filled the plates. The maximum temperature for growth of the kudzu Phytophthora isolates and P. erythroseptica were also examined under 33°C, 35°C, 37°C, and 39°C for comparison. Pathogenicity of KACC47617 to 47619 was tested and confirmed as described ahead for KACC47616.

tDNA internal transcribed spacer (ITS) and cytochrome oxidase subunit 1 (COXI) gene of the four isolates of Phytophthora sp. were sequenced and compared with similar species in clade 7 of Phytophthora. The sequences were analyzed by using Tamura-Nei parameter distance calculation model, which was then used to construct the neighbor-joining tree with Mega version 5 (Tamura et al., 2011). All kudzu isolates grew well on V8 and WA media. Mycelia were hyaline, coenocytic, mature mycelia were about

Fig. 1. Symptoms on leaves and vine of kudzu (Pueraria lobata (Willd.) Ohwi) (A–C) and on leaflets of black locust (Robinia pseudo-acacia L.) (D).

Fig. 2. Coralloid mycelium (A) and hyphal swelling (B) of Phytophthora sp. causing leaf blight and vine rot of kudzu (KACC47617). Scale bar=20 μm.
5–7.5 μm thick and often became irregular coralloid, and abundant catenulate hyphal swellings (Fig. 2), 15–35 μm in diameter, were found in most cultures. No chlamydospore production was observed. All isolates produced abundant sporangia on V8 agar blocks put in SDW while KACC 47617, KACC 47618, and KACC 47619 also produced sporangia in WA. The three fungal isolates, KACC 47617, KACC 47618, and KACC 47619, readily produced sex organs on V8 medium showing homothallic nature while KACC 47616 did not produce any sex organs on any media even though it was old, so possibly it was sterile or heterothallic. Sporangia and sex organs were slightly varying in size depending on isolates and culture media. The dimensions of the sporangia and sex organs are shown in Tables 1 and 2, respectively. Sporangia ranged from subspherical, ovoid, obovoid, ellipsoidal, limoniform, fusiform, pyriform to distorted shapes and were terminal, nonpapillate with inconspicuous apical thickening (Fig. 3). New sporangia were produced by internal extended and nested proliferation and external proliferation was also observed (Fig. 4). Sporangia were measured at 20–70×15–45 μm with length/breadth ratio of 1.3–1.8. Zoospores were 10–20 μm in diameter and discharged through the openings of sporangia about 6.3 to 17.5 μm wide (Table 1). Oogonia were spherical and 25–47.5 μm in diameter with the average of 36.3 μm (Table 2). Oospores were round, hyaline to yellowish brown, aplerotic and 18.75–40 μm in diameter. Antheridia were amphigynous, round to ovoid and ranging in 10–28.8 μm in length and 10–22.5 μm in breadth (Fig. 5).

The kudzu isolates showed mycelial colony with a smooth to light stellate pattern on V8 and PDA media (Fig. 6). Optimum and maximum temperature for kudzu isolates were 25–30°C and 37°C, respectively and did not grow at 39°C. On the other hand, *P. erythroseptica* did not grow at 35°C as

| Isolates   | Sporangia (V8 water) | Sporangia (WA blocks) | Sporangia opening (μm) | Zoospore diameter (μm) |
|------------|---------------------|-----------------------|------------------------|------------------------|
|            | Length (μm)         | Breadth (μm)          | L:B                    | Length (μm)            | Breadth (μm)          |                           |
| KACC47616  | 20–66.3 (48.0±10.9) | 15–45 (32.0±7)       | 1.5                    | 10–17.5                | 10–15                 |
| KACC 47617 | 35–67.5 (50.4±7.6)  | 20–40 (31.6±5.8)     | 1.6                    | 20–47.5                | 17.5–38.8             | 1.3 6.3–13 10–20 9.6±1.5 12.8±2.3 |
| KACC47618  | 33.8–70 (46.3±8.1)  | 13.8–40 (26.0±5.1)   | 1.8                    | 27.5–60                | 21.3–38.8             | 1.5 10–16.3 11.3–15 13.4±1.7 13.6±1.3 |
| KACC47619  | 25–65 (45.4±9.1)    | 15–37.5 (27.5±5.3)   | 1.7                    | 20–70                  | 15–47.5               | 1.5 10–15 10–16.3 12.5±1.7 12.8±1.3 |

Values are presented as range (mean±standard deviation).

| Isolates   | Oogonium diameter (μm) | Oospore diameter (μm) | Antheridium Length (μm) | Antheridium Breadth (μm) |
|------------|------------------------|-----------------------|-------------------------|-------------------------|
| KACC47616  | -                      | -                     | -                       | -                       |
| KACC 47617 | 30–47.5 (39.8±3.4)     | 26.3–42.5 (34.2±3.1)  | 13.8–23.8               | 11.3–20                 |
| KACC47618  | 26.3–43.8 (34.75±3.3)  | 20–33.8 (28.58±2.6)   | 10–28.8                 | 10–22.5                 |
| KACC47619  | 25–45 (34.3±4.4)       | 18.75–40 (28.7±4.1)   | 12.5–25                 | 10–20                   |

Values are presented as range (mean±standard deviation).
reported by Ho and Jong (1989) (Fig. 7).

The phylogenetic trees constructed by ITS rDNA and COXI sequence showed that the kudzu isolates had identical sequences with those of the black locust isolates, \textit{P. cinnamomi} var. \textit{robiniae} (Figs. 8, 9).

\textit{Phytophthora} sp. which infected black locust was reported in China as \textit{Phytophthora cinnamomi} var. \textit{robiniae}. The morphological characteristics of the \textit{Phytophthora} sp. isolated from kudzu plants in the present study were very similar with the black locust isolates reported by Ho et al. (1983) and Ho (2002). On the basis of morphological and molecular characteristics, the kudzu strains in this study could be concluded to be identical to \textit{Phytophthora cinnamomi} var. \textit{robiniae} reported by Ho (2002).

Rahman et al. (2014) reported that two \textit{P. cinnamomi} var. \textit{robiniae} isolates from black locust showed 100% sequence identity in rDNA ITS with kudzu isolates which were found in the Toyama and Ishikawa districts of Japan. They also
found that the morphological characteristics of the black locust isolates, *P. cinnamomi* var. *robiniae*, were very similar to those of the kudzu isolates found in Japan. Therefore, they have classified both the kudzu and black locust isolates as the new species, *P. asiatica*.

In this study, as shown in Table 3 which compares the morphologies of three isolates, *P. cinnamomi* var. *robiniae*, black locust isolates, *Phytophthora* sp. infecting kudzu plants in Korea and *Phytophthora asiatica* which infected kudzu plants in Japan, all isolates are very similar to each other and the Korean kudzu isolates have many common similarities with two other isolates, black locust isolates and Japan kudzu isolates. In addition, findings of the ITS rDNA and COXI sequence in this study and phylogenetic trees reported by Rahman et al. (2014) also agreed that all isolates were quite similar. Therefore, all kudzu isolates from Korea are similarly identified as *Phytophthora asiatica*.
Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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Table 3. Comparison of three isolates: black locust isolates, Korean kudzu isolates and Japan kudzu isolates

| Descriptions                  | P. cinnamomi var. robiniae on black locust (Ho, 2002) | Phytophthora sp. on kudzu in Korea | P. asiatica on kudzu in Japan (Rahman et al, 2014) |
|-------------------------------|-------------------------------------------------------|-----------------------------------|-----------------------------------------------------|
| Hyphal swelling              | Catenulate to reticulate                              | Catenulate                        | Catenulate, formed in water                          |
| Chlamydospore                | Not observed                                          | Not observed                       | Not observed                                        |
| Sporangiochore                | Single or simple sympodial                            | Single or simple sympodial        | Single or simple sympodial                           |
| Sporangia                    | Terminal, ovoid or obpyriform                         | Terminal, subspherical, ovoid, obovoid, ellipsoid, limoniform, fusiform, pyriform to distorted shapes | Terminal, ellipsoid, obturbinete, obpyriform, distorted |
| Size (μm)                    | 40–70×24–41                                          | 20–70×15–45                       | 27–72×15–44                                        |
| L:B ratio                    | 1.3–2                                                 | 1.5–1.7                           | 1.6                                                 |
| Papilla                      | Nonpapillate                                          | Nonpapillate                       | Nonpapillate                                        |
| Proliferation                | Internal, both nested and extended or external        | Internal, both nested and extended or external | Internal, both nested and extended                   |
| Caducity                     | Noncaducous                                           | Noncaducous                        | Noncaducous                                         |
| Sexual system                | Heterothallic but occasionally self-fertile          | Homothallic and rarely may be heterothallic | Homothallic                                         |
| Oogonia                      | Smooth                                                | Smooth                             | Smooth                                             |
| Shape                        | Globose or pyriform                                   | Globose or pyriform                | Spherical                                          |
| Size (μm)                    | 30–43                                                 | 39.8±3.4                          | 40±8.3                                             |
| Oospore                      | Aplerotic                                             | Aplerotic                          | Aplerotic                                          |
| Total range (μm)             | 28–37                                                 | -                                 | 33±7.1 (15–43)                                      |
| Antheridium                  | Amphigynous                                           | Amphigynous                        | Predominantly amphigynous                           |
| Total range (μm)             | 13–19×13–16                                          | 10–28.8×10–22.5                   | 13–28×8–18                                         |
| Optimum temperature (°C)     | 28–32                                                 | 25–30                             | 28                                                 |
| Maximum temperature (°C)     | 36–37                                                 | 37                                | 35, no growth at 40                                 |

L:B ratio, length:breadth ratio.

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