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First report of *Sclerotium rolfsii* in the Lao PDR

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Abstract In May 2010 basal stem rot of snake bean (long bean) (*Vigna unguiculata* subsp. *sesquipedalis*) caused by *Sclerotium rolfsii* was discovered in Vientiane Capital, Lao PDR, during an *ad hoc* disease survey. The disease had resulted in death of some infected plants. The basal stem region had a bleached appearance, a typical symptom of this disease. Abundant small, round, brown sclerotia were present on the stem base, and on the adjacent soil and dead leaf material. The fungus was isolated into pure culture and Koch's postulates were fulfilled. This is the first report of *S. rolfsii* in the Lao PDR, and the first report of basal stem rot of snake bean caused by this pathogen in the Lao PDR.

Keywords *Sclerotium rolfsii* · Basal stem rot · *Vigna unguiculata* subsp. *sesquipedalis* · Snake bean · Long bean

Sclerotium rolfsii is a common soil-borne fungal plant pathogen of tropical, sub-tropical and warm temperate regions, usually infecting the stem base region of a wide range of host plants (Aycock 1966; Punja 1985). It is, for example, common in Vietnam, a country adjacent to the Lao PDR (Burgess et al. 2008). *S. rolfsii* typically forms abundant white mycelium and small, brown, round sclerotia on diseased tissue under hot humid conditions, and may spread over the soil surface from a nutrient base such as a diseased stem base, diseased pods and leaf residue.

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We discovered a basal stem rot of snake bean (long bean) (*Vigna unguiculata* subsp. *sesquipedalis*) (Fig. 1) typical of that caused by *S. rolfsii*, in Xiengda Village, Saysetha District, Vientiane Capital, Lao PDR in May 2010. It caused death of plants (Fig. 2) and the infected basal stem region had a bleached appearance (Fig. 3), a typical symptom of this disease. Abundant small, round, brown sclerotia, characteristic of *S. rolfsii*, were present on the stem base of diseased plants (Fig. 3), on the adjacent soil and dead leaf material, and rice hulls used as mulch around the plants.

We collected samples of diseased stem bases for isolation of the putative pathogen. The stem sections were washed in tap water to remove dust before swabbing with 70 % ethyl alcohol (ETOH).

The sections were then scraped aseptically to remove a thin outer layer of stem tissue and swabbed lightly again with ETOH. Thin transverse sections of stem tissue were then removed aseptically and plated on carnation leaf-piece agar (CLA) and potato carrot agar (PCA). Colonies that developed from the sections were sub-cultured to water agar to facilitate hyphal tipping (Burgess et al. 2008) to produce pure cultures on potato dextrose agar (PDA) for identification. The purified cultures were typical of *S. rolfsii* with white mycelium, and abundant small, round, brown sclerotia ranging in diameter from 0.5 to 2.0 mm, formed after 10 day (Burgess et al. 2008; Punja and Damiani 1996). A representative culture was accessioned as LPP9 in the living culture collection of the Plant Pathology Unit of the Plant Protection Centre in Vientiane. A sub-culture of LPP9 (= CPC 19158 = CBS 132553) was forwarded to the Centraalbureau voor Schimmelcultures (CBS) in the Netherlands for confirmation of identification and accessioning.

Genomic DNA was extracted from fungal colonies growing on MEA using the UltraClean™ Microbial DNA Isolation Kit (MoBio Laboratories, Inc., Solana Beach, CA, USA) according to the manufacturer's protocol. The primers V9G (de Hoog and Gerrits van den Ende 1998) and

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Fig. 1 Healthy snake bean plant with typical long snake-like pod

Fig. 2 Two wilted snake bean plants affected by basal stem rot caused by *Sclerotium rolfsii*, the result of natural infection

LR5 (Vilgalys and Hester 1990) were used to amplify part (ITS) of the nuclear rDNA operon spanning the 3' end of the 18S nrRNA gene, the first internal transcribed spacer (ITS1), the 5.8S nrRNA gene, the second ITS region and the 5' end of the 28S nrRNA gene. The primer ITS4 (White et al. 1990) was used as internal sequence primer to ensure good quality sequences over the entire length of the ITS region. A mega-blast search of the NCBI's GenBank nucleotide database using the ITS sequence generated in this study (GenBank JX566993) revealed 100 % identity over 621 nucleotides to publicly available sequences of *Athelia rolfsii* (GenBank AB042626, anamorph *Sclerotium rolfsii*) and *Sclerotium delphinii* (GenBank JN241565 and JN241567, synonym *Sclerotium rolfsii* var. *delphinii*). The ITS sequence differed with one nucleotide from additional sequences of *Athelia rolfsii* (GenBank JN543691, JN017198 and AB075298).

A pathogenicity test was undertaken using 14-day-old snake bean seedlings grown in potting mix (mushroom compost:sawdust:sand, 1:1:1 by volume) in pots at air temperatures varying from 27 to 34 °C. The mushroom compost and sawdust were well decomposed and thoroughly colonised by microorganisms thus simulating a competitive soil microflora. This particular potting mix was used because pasteurised field soil was not available. Culture LPP9 was used to colonize 3 cm long sections of snake bean stems, by inoculating PDA in a 9 cm diam glass Petri plate (Fig. 4). The stem sections had been sterilized by autoclaving for 30 min. The stem sections were thoroughly colonized by *S. rolfsii* after 7 day and some sclerotia had formed on the surface of the sections. They were then air-dried and used to inoculate the snake bean seedlings by placing a colonized

Fig. 3 Basal stem rot caused by *Sclerotium rolfsii* of two plants shown in Fig. 2 – *arrows* indicate small sclerotia

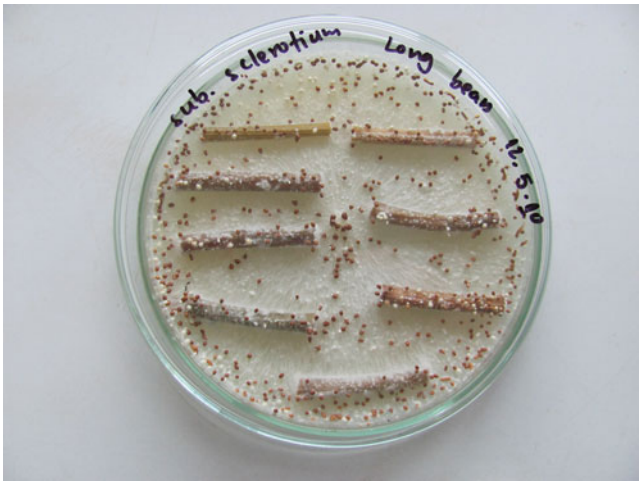

Fig. 4 Sterile stem sections of snake beans being colonized by Sc*lerotium rolfsii* in pure culture, for use in pathogenicity test

stem section on one side of the stem base of each of two plants in each of four pots. The colonized stem section was held against the stem base with a wooden toothpick (Fig. 5). A further eight plants were slightly wounded by pricking the side of the stem at the soil line with a sterile needle to simulate insect damage, before a colonized stem section was placed against the wound site. Sterile stem sections were placed against eight non-wounded, and eight wounded plants. Basal stem rot developed after 6 day in inoculated non-wounded and wounded stems, and diseased plants wilted rapidly with no symptoms of leaf chlorosis (yellowing). *S. rolfsii* was re-isolated using the same procedures as described above, fulfilling Koch's postulates. The infection of non-wounded stems indicates that the fungus is a primary pathogen able to infect non-damaged tissue. Snake beans are listed as a host of *S. rolfsii* in the review by Aycock (1966), presumably causing a basal stem rot.

Fig. 5 Inoculation of snake beans with stem sections colonized by *Sclerotium rolfsii*. The growth of *S. rolfsii* over the soil surface is typical of the growth commonly observed in the field

Dried colonized snake bean stems were used as the source of inoculum as this pathogen persists as hyphae in old infested stem tissue on and in soil as well as sclerotia in this tropical region where crops are grown in short rotations. Infested stem residues commonly remain on the soil surface and the stem base of the next crop makes contact with old infested residue that presumably provides a food base to enhance infection. Consequently the inoculum was placed on the soil surface in the pathogenicity test. Furthermore the location of inoculum on the soil surface is appropriate because *S. rolfsii* normally infects the stem base. There was no evidence of root infection of the snake beans by this pathogen. This finding is in agreement with observations on infection of various hosts grown in a similar climate in Vietnam over many years. It is likely that sclerotia are also responsible for the infection of some plants in the field in this region.

This is the first report of *S. rolfsii* in the Lao PDR. It is also the first report of basal stem rot of snake beans caused by *S. rolfsii* in the Lao PDR. The authors have also observed *S. rolfsii* associated with a base rot on garlic and a head rot of cabbages in the Vientiane area (authors' unpublished data) and isolated the fungus into pure culture. Further surveys are needed over several seasons to assess the incidence and economic importance of basal stem rot of snake beans, and the presence and incidence of diseases of other crops caused by *S. rolfsii*, in the Lao PDR.

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