First report of *Rhizoctonia solani* anastomosis group AG-4 HG-I in the Lao PDR

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Abstract *Rhizoctonia solani* anastomosis group AG-4 HG-I is reported for the first time from the Lao PDR. It was isolated from gai lan (*Brassica oleracea* var. *alboglabra*) affected by collar rot, seedling death, root rot and stunting of older plants from the Paksong area of Champasak province. The anastomosis group was confirmed by sequencing and Koch's postulates were fulfilled.

Keywords Chinese broccoli · Chinese kale · Kai lan · *Rhizoctonia* collar rot · Wirestem

Rhizoctonia solani is a species complex consisting of a number of anastomosis groups (AGs) that differ morphologically and phylogenetically with respect to host range and pathogenicity, susceptibility to different fungicides and geographic distribution (Gonzalez et al. 2001; González et al. 2006; Vilgalys and Cubeta 1994). They persist as hyphae in residue and/or sclerotia in soil and affect many crops, causing a wide range of diseases including foliar spots and blights, seedling death (damping-off), collar rot, root rot, crater rot, stalk and

head rots of Brassica and other crops (Rimmer et al. 2007; Sneh et al. 1996). It is important to identify the AG causing a particular disease in order to determine the most effective integrated disease management (IDM) strategy and to contribute to the global understanding of the distribution and biology of *R. solani*.

A high incidence of collar rot (wirestem) was observed in seedlings and older plants of gai lan (Chinese flowering broccoli (*Brassica oleracea* var. *alboglabra*)) grown in ground beds (natural soil) in a polyhouse at a farm in Paksong District, Champasak province, Lao PDR, in August 2013 (Fig. 1). Discrete root rot lesions along the roots and at the root tips were also observed in older plants. The collar rot caused death of seedlings. Collar rot and root lesions were associated with severe stunting of older plants.

Samples of diseased seedling stems were collected for isolation of the putative pathogen. The stem sections were washed gently in sterile water, immersed briefly (1 s) in 70 % ethyl alcohol (ETOH), immediately rinsed in sterile water, and then damp-dried on sterile paper towel. Ten stem sections (2–3 mm long) were removed aseptically from the junction of diseased and symptomless tissue, plated on Water Agar (WA) amended with 0.2 g/L penicillin and 1 g/L streptomycin sulfate and incubated at room temperature. The hyphal growth that developed from the stem sections was morphologically similar to that of *R. solani*, with obvious right-angled branching and a constriction at the base of hyphal branches (Fig. 2). Colonies that developed from three sub-cultures on PDA were brown pigmented, produced dark brown sclerotia and were morphologically typical of *R. solani*. A representative colony was purified by hyphal tipping (Burgess et al. 2008) and forwarded to the International Collection of Micro-organisms from Plants (ICMP), Landcare Research, Auckland, New Zealand for identification of AG by sequencing ITS-rDNA. It was accessioned as ICMP 20043 and preserved cryogenically in liquid nitrogen: <http://scd.landcareresearch.>

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Fig. 1 Collar rot symptoms, typical of *Rhizoctonia solani* infection, observed on gai lan (*Brassica oleraceae* var. *alboglabra*) in ground beds in Paksong. **a** Stunted and wilting gai lan patch (centre of image), surrounded by healthy plants and sites where seedling death has occurred and weed growth is occurring; **b** Collar rot and lack of lateral root development symptoms on an infected plant

co.nz/Specimen/ICMP_20043. The same isolate was tested for pathogenicity.

DNA was extracted from mycelium using a Roche REDExtract-N-Amp Plant PCR Kit. The ITS-rDNA region was amplified using the ITS1F and ITS4 PCR primers following the protocol of Weir et al. (2012), and the amplicon was sequenced on a ABI 3500xl sanger sequencer. The sequence was compared to reference sequences of *R. solani*

Fig 2 Right-angled branching and constriction at the base of hyphal branches (in water) of isolate ICMP20043, typical of *Rhizoctonia solani*

anastomosis groups in GenBank and was found to be most similar to group AG-4 HG-I at 99.5 to 96 % identity, compared to group AG-4 HG-II at 94 to 93 % identity and group AG-4 HG-III at 88 to 87 % identity. The ITS sequence from isolate ICMP 20043 was deposited in GenBank as KM013470. Reference sequences of the three *R. solani* AG-4 subgroups (HG-I, HG-II and HG-III) were downloaded from GenBank and a multiple alignment constructed with Geneious 7.1.5 (<http://www.geneious.com>). A phylogenetic analysis was run in MrBayes 3.2.2 (<http://mrbayes.sourceforge.net>) for 2.2 million generations with a 10 % burnin and with flat prior probabilities (Fig. 3). Diagnostics using Tracer 1.5 (<http://tree.bio.ed.ac.uk/software/tracer/>) indicated that the analysis had reached convergence.

A pathogenicity test was undertaken using 9-day-old seedlings of gai lan grown in cells in a plastic seedling tray with four seeds sown per cell. The soil was a mix of two parts (by volume) alluvial soil, which had had not been cropped previously to vegetables, and one part clean river sand. The alluvial soil was used to provide a competitive soil microflora. The inoculum was prepared using a procedure adapted from Songvilay et al. (2013). Culture ICMP 20043 was sub-cultured onto PDA in 9 cm diam glass Petri plates and sterile rice hulls were placed on the medium. The rice-hulls were thoroughly colonized by *R. solani* after 14 days. Four colonized rice hulls were placed on the soil surface adjacent to each seedling that had emerged in each of four cells (Fig. 4a). Sterile rice-hulls were added to four control cells. The seedling tray was then covered with a moistened plastic bag for 24 h to provide a humid environment similar to that in the field and maintained at 25 to 30 °C. Collar rot at the soil line and seedling wilting and collapse were observed from 1 day post-inoculation (Fig. 4b). Diseased seedlings wilted quickly with no symptoms of leaf chlorosis (yellowing). All seedlings had wilted and/or collapsed by 4 days after inoculation. The control seedlings did not develop symptoms. *Rhizoctonia solani* was re-isolated using the same procedures as described above, fulfilling Koch's postulates.

The recovery of AG-4 HG-I from gai lan is consistent with the reports of the AG-4 group causing disease in *B. oleracea* and *B. napus* in North America (Keinath and Farnham 1997) and in cauliflower in Belgium (Pannecouque et al. 2008). More recently, AG-4 HG-II was isolated from *B. oleracea* crops in the UK during an extensive survey (Budge et al. 2009). We have observed collar rot of seedlings and older plants and seedling death in several brassicas in Champasak province. Consequently, further studies are needed to determine the relative importance of AG-4 HG-I in brassica crops in this region.

We have also observed collar rot on other vegetable crops at the polyhouse farm where AG-4 HG-I was recovered, and at other farms in the province, indicating the possible presence of additional hosts for AG-4 HG-I and of other anastomosis

Fig 3 A 50 % majority-rule Bayesian inference phylogenetic tree of ITS gene sequences showing the *Rhizoctonia solani* AG-4 groups. Tip labels are GenBank accession numbers, the isolate of this study ICMP 20043 is indicated in bold font. Node labels indicate posterior probability support. The tree was rooted with an AG-1 1C isolate as an out-group

Fig. 4 Pathogenicity test (a) Inoculation with rice hulls colonised by *Rhizoctonia solani*, (b) Collar rot symptom development at 1 day after inoculation, showing collapse at the soil line (indicated by white arrows) and (c) Collar rot and root tip dieback symptoms (indicated by black arrows) at 1 day after inoculation

groups of *R. solani*. The high incidence of *Rhizoctonia* collar rot and seedling death in gai lan is attributed to the current practice of raising seedlings in natural soil in ground beds in polyhouses where brassica crops, including gai lan, have been grown regularly for at least seven years. The production of seedlings in pathogen-free soil is being encouraged together with regular rotation to crops in other plant families. Besides infecting vegetables in the Brassicaceae, *R. solani* AG-4 is known to have a wide host range, infecting crops in the Chenopodiaceae, Fabaceae and Solanaceae (Ogoshi 1987) and Cucurbitaceae (Kuramae et al. 2003). Limited but potentially suitable rotation vegetable crops should be tested for susceptibility to AG-4 HG-I in the Alliaceae, Apiaceae and Asteraceae.

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