CHARACTERIZATION OF THE WEB BLIGHT PATHOGEN INFECTING WILD BEANS IN THE REMOTE AREAS OF NORTHERN ARGENTINA.

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Web blight (WB) of common bean (Phaseolus vulgaris L) is a yield-limiting disease in Central America and the Caribbean (Galvez et al., 1989). The disease is caused by aerial isolates of the fungus Rhizoctonia solani Kuhn [teleomorph: Thanatephorus cucumeris (Frank) Donk]. R. solani is a complex species whose current classification is based largely on placing isolates into anastomosis groups (AG). At least 13 AG have been described within R. solani. Many AG have been subdivided further into subgroups that differ for one or more genetic, biochemical, molecular or pathogenic characteristic (Sneh et al., 1991). Isolates that cause WB of common bean represent at least four subgroups of R. solani (Godoy-Lutz et al., 1996).

Botanical collections of wild beans Phaseolus vulgaris aboragineus (Pva) conducted in 1995-1997 have identified leaves with characteristic symptoms of WB. These samples have been collected from isolated areas in the middle altitude Andean rainforest of Argentina where no small landholder crops are grown within 10 km. and no large commercial beans are within 50 km. WB has been reported on cultivated beans in the northwestern region of Argentina but has not been reported to infect wild or weedy beans. The objective of this study was to identify and characterize the pathogen isolates associated with WB symptoms in Pva by traditional and molecular techniques and to determine their virulence on commercial bean lines/cultivars.

Wild bean (Pva) leaves were collected at 1363-1740 masl in Calilegua National Park, Valle Grande and the Salta province of Argentina during a search for indigenous bean plants and associated plant pathogens. The margins of leaf lesions with WB symptoms were removed and plated directly on water agar. Rhizoctonia-like colonies were then transferred to Difco potato dextrose agar for AG-typing and determination of cultural characteristics (Sneh et al., 1991). Subgroup determination was made by analysis of the internal transcribed spacers (ITS) of nuclear ribosomal DNA repetitive units. Genomic DNA of fungal isolates was extracted from lyophilized mycelium. Primers ITS 1 and ITS 4 were amplified by polymerase chain reaction (PCR). Restriction patterns of the ITS region were generated with enzymes Eco RI, Msp1 and Hae III using the procedures of Liu and Sinclair (1992).

Virulence of the isolates was determined in the greenhouse by inoculation of cotyledons of two-week-old seedlings of commercial cultivars Alubia and PC-50 (Andean origin) and DOR-500 (Middle American origin) by a method previously reported (Godoy et al., 1996).

We recovered 28 isolates of R. solani AG-2 group associated with WB symptoms typical of basidiospore infection. Isolates selected for restriction fragment length polymorphism (RFLP) analysis showed similar banding patterns with restriction enzymes Eco RI and Msp 1 but showed a different pattern with Hae III. These isolates were separated into two groups on the basis of fragment banding pattern generated with Hae III. Isolates A1, A4 and A26-1 exhibited a banding pattern similar to the anastomosis tester isolate R-135 and the commercial bean isolate EEAL-A
and were assigned to AG-2-2 IV. Isolates A8, A18, A19, A24, A27, and A28 were assigned to AG-2 unknown since their banding pattern did not fit any previously described interspecific group (Liu and Sinclair, 1992; Kanematsu and Naito, 1995).

All isolates inoculated on DOR 500, PC-50 and Alubia caused water soaked lesions after three days incubation. The lesions were similar in size to those caused by the field isolate EEAL-A from the Dominican Republic.

This is the first report of isolates of R. solani AG-2 infecting wild beans and in particular those from the rainforest of the Argentinian Andes. AG-2 isolates cause WB in commercial bean varieties in Colombia, Dominican Republic, Panama and Costa Rica (Galvez et al., 1989; Godoy et al., 1996).

Collectively, isolates of AG-2 have been divided into six subgroups by RFLP analysis of PCR amplified nuclear ribosomal DNA in the ITS region. (Liu and Sinclair, 1992; Kanematsu and Naito, 1995). The genetic variability within the AG groups is derived from fungal mechanisms such as sexual recombination and heterokaryosis and enhances ecological adaptation and survival of the pathogen. In general, isolates within subgroups are believed to have a narrow host range, however, in recent years new virulent forms of soilborne R. solani AG-2 have been reported to be pathogenic to two or more unrelated crops (Liu and Sinclair, 1992). Therefore, knowledge of the variability of WB pathogen populations is a prerequisite to reducing pathogen dissemination and implementing effective disease management strategies.

Subgroups of R. solani AG-2 are genetically stable independent populations and are widespread over very broad geographical regions according to Liu and Sinclair (1992). However, their study did not include R. solani isolates from common bean or from the Americas outside the USA. The similarities in RFLP band patterns observed between isolates R-135 and EEAL-A and the rainforest isolates A1, A4 and A26-1 support the concept of stable, widespread independent populations. More information about pathogen variability, host range, inoculum survival and spread of AG-2 isolates causing WB of common beans, both wild and domesticated is needed.

REFERENCES