

Basal Stem Rot of Broccoli Caused by *Rhizoctonia solani* AG-2-1 and AG-4 in Korea

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ABSTRACT

Basal stem rot symptoms were frequently observed on broccoli plants grown in farmers' fields located in Gimje and Jeju, Korea during disease surveys carried out in November and December 2019. Incidence of the diseased plants was 1-10% in the fields investigated. Five isolates of *Rhizoctonia* sp. were obtained from the diseased plants. Of the isolates, two were identified as *Rhizoctonia solani* AG-2-1 based on the morphological characteristics and anastomosis test, and three were as *R. solani* AG-4. Two isolates each of *R. solani* AG-2-1 and AG-4 were tested for pathogenicity on broccoli by artificial inoculation. All the tested isolates induced basal stem rot symptoms on the inoculated plants. The symptoms were similar to those observed in the fields investigated. This is the first report of basal stem rot of broccoli caused by AG-2-1 and AG-4 in Korea.

Keywords: Anastomosis Group; Basal Stem Rot; Broccoli; Pathogenicity; *Rhizoctonia solani*

Introduction

Broccoli (*Brassica oleracea* L. var. *italica* Plenck) is a popular vegetable grown worldwide. The vegetable is also widely grown in Korea. Basal stem rot symptoms were frequently observed on broccoli plants grown in farmers' fields located in Gimje and Jeju, Korea during disease surveys carried out in November and December 2019. The authors collected diseased plant samples from the broccoli fields and isolated fungi to determine the cause of the disease. *Rhizoctonia* sp. was most frequently isolated from the diseased plant samples. *Rhizoctonia* spp. are identified based on the morphological characteristics and anastomosis test [1,2]. Of the *Rhizoctonia* spp., *Rhizoctonia solani* Kühn has the most strains and hosts compared to other species. Many anastomosis groups are reported to exist in *R. solani* and known to have different genetic and pathological characteristics [2]. Anastomosis groups of *R. solani* isolates are identified by hyphal fusion reaction with tester isolates. In this study, identification of *Rhizoctonia* sp. isolates from diseased broccoli plants was carried out based on the morphological characteristics and anastomosis test. In addition, pathogenicity of the isolates was investigated by inoculation test.

Materials and Methods

Field Survey and Collection of Samples

Broccoli fields were surveyed at locations Gimje and Jeju in Korea in November and December 2019. Three sites were observed in the field, and 100 plants at each site were investigated for disease incidence. The diseased broccoli plants were collected to isolate causal fungus.

Isolation

Nine to 15mm² lesion pieces cut from the diseased plant parts were plated on 2% water agar (WA) after surface-sterilizing with 1% sodium hypochlorite solution for one minute. Fungi were isolated from the lesion pieces after one to two days of incubation at 25 °C. The fungal isolates were transferred to potato dextrose agar (PDA) slants and used for identification and pathogenicity test.

Investigation of Morphology and Anastomosis Test

Rhizoctonia sp. isolates obtained from the diseased plants

were cultured on PDA in 9cm-diameter petri dishes at 25 °C for 10 days and examined for the morphological characteristics. The *Rhizoctonia* sp. isolates were tested to classify anastomosis groups using tester isolates of *R. solani* as previously described [3]. The *R. solani* tester isolates AG-1 (KACC 40101), AG-2-1 (KACC 40119), AG-2-2 (40125), AG-3 (KACC 40138), AG-4 (KACC 40139), and AG-5 (KACC 40146) were obtained from the Agricultural Microbiology Division, National Institute of Agricultural Sciences in Korea.

Pathogenicity Test

Broccoli seeds were sown in circular plastic pots (height, 9cm; upper diameter, 10cm; lower diameter, 7cm) filled with soil in the greenhouse at 20-28 °C. Twenty six-day-old plants of broccoli grown in the pots were used for inoculation tests. Selected isolates of *Rhizoctonia* sp. were tested for their pathogenicity on the broccoli plants. Mycelial disks of 6mm in diameter were cut from the margins of actively growing cultures of each isolate on PDA and placed at the soil surface level of the broccoli plants. Inoculated plant pots were placed in plastic boxes (60cm × 43cm × 33cm) with

100% relative humidity at room temperature (24-26 °C) for two days. Then, the inoculated plant pots were removed from the boxes and kept indoors. The virulence of the isolates was rated based on the degree of basal stem rot symptoms five days after inoculation. The inoculation test was performed in three replicates.

Results

Symptoms and Disease Incidence

Basal stem rot symptoms were frequently observed on broccoli plants grown in the fields investigated. The symptoms began on the plant parts at the soil surface. The diseased plants wilted and blighted in the early stage of the plant growth (Figures 1A & 1B). The basal stem of the infected plant receded and was thin, discolored, and rotten (Figure 1C). The diseased plants were retarded in their growth and fell down in the medium stage of the plant growth (Figure 1D). The basal stems of the diseased plants turned dark brown and rotten (Figure 1E). The incidence of diseased plants in the fields investigated at two locations in Korea during the disease surveys was 1-10% (Table 1).



Figure 1: Basal stem rot symptoms of broccoli plants.

A-C: Symptoms in the early stage of the plant's growth observed in the field;

D and E: Symptoms in the medium stage of the plant's growth observed in the field;

F: Symptoms (left, AG-2-1 inoculated; center, AG-4 inoculated; right, non-inoculated) induced by artificial inoculation tests with *R. solani* isolates.

Table 1: Incidence of basal stem rot on broccoli plants in the fields located in Gimje and Jeju in Korea in 2019.

Location Investigated	Month Investigated	No. of Fields Investigated	No. of Fields with Disease	% Diseased Plants*
Gimje	November	1	1	1-5
Jeju	December	2	2	1-10

*Three sites were observed in the field, and 100 plants at each site were investigated.

Isolation and Identification

Five isolates of *Rhizoctonia* sp. were obtained from basal stems of the diseased plants. All the isolates were identified as *R. solani* based on the morphological characteristics as per descriptions from previous studies [1,2]. Of five isolates of *R. solani*, two were classified as *R. solani* AG-2-1 by anastomosis test, and three as *R.*

solani AG-4. Anastomosis reactions between the tested isolates and the tester isolates of *R. solani* AG-2-1 and AG-4 observed by light microscope are shown in Figures 2A & 2B. The colony of *R. solani* AG-2-1 cultured on PDA showed brown concentric zones (Figure 2C). The colony of *R. solani* AG-4 cultured on PDA were whitish light brown, and sclerotia were not produced on the medium (Figure 2D).

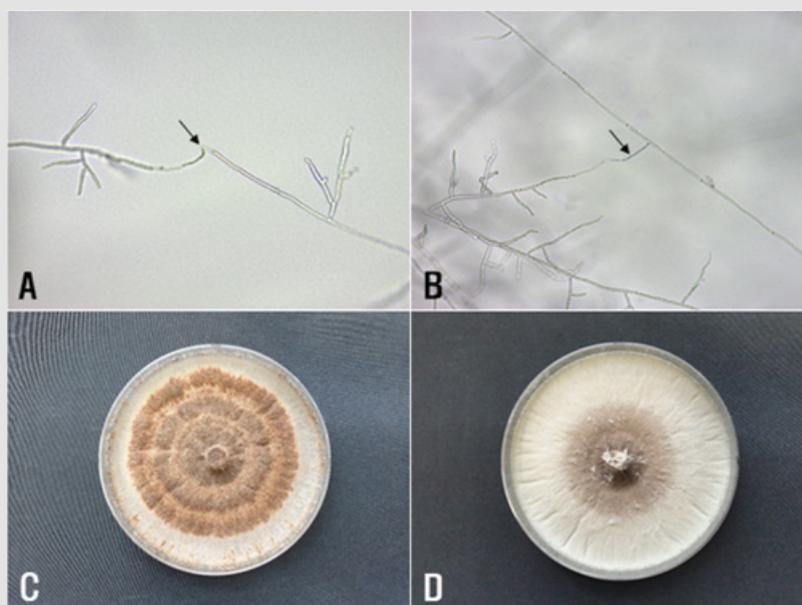


Figure 2: Anastomosis test of *Rhizoctonia solani* isolates from broccoli and cultural appearance of the isolates.

A and B: Anastomosis reactions between the tested isolates (left) and the tester isolates (right) of *R. solani* AG-2-1 and AG-4 observed by light microscope, respectively. The arrows indicate points of hyphal anastomosis;

C and D: Colonies of *R. solani* AG-2-1 and AG-4 isolates grown on PDA at 25 °C for 10 days, respectively.

Pathogenicity

Two isolates each of *R. solani* AG-2-1 and AG-4 were tested for pathogenicity on broccoli by artificial inoculation. All the tested isolates of the two anastomosis groups induced basal stem rot on

the inoculated plants, whereas no symptom was observed on the control plants (Table 2 & Figure 1F). The symptoms induced by the artificial inoculation of the plants were similar to those observed in plants from the fields investigated. The isolates which induced symptoms on the plants were re-isolated from the lesions.

Table 2: Pathogenicity of *Rhizoctonia solani* AG-2-1 and AG-4 isolates on broccoli plants by artificial inoculation.

Anastomosis Group	Isolate No.	Location Source of Isolates	Virulence of Isolates*
AG-2-1	RS1933	Gimje	+
	RS1934	Gimje	+
AG-4	RS1930	Jeju	+
	RS1931	Jeju	+
Control			-

*Disease severity was rated five days after inoculation. + : severe basal stem rot induced, - : no symptom.

Discussion

R. solani is reported to cause various diseases such as damping-off, seedling rot, stem and root rot, wire stem, basal stem rot, etc. in many crops [4-6]. It has been reported that *R. solani* AG-2-1 and AG-2-2 VI cause damping-off and foot rot of broccoli in Japan, respectively [7,8]. However, there has been no report of basal stem rot on broccoli caused by *R. solani* AG-2-1 and AG-4. This is the first report of basal stem rot of broccoli caused by AG-2-1 and AG-4 in Korea.

Anastomosis groups AG-1 through 13 of *R. solani* have been reported, and some anastomosis groups of *R. solani* are further divided into subgroups by cultural type, pathogenicity, DNA-DNA hybridization, rDNA-ITS sequence analysis, etc. [9]. It is reported that *R. solani* AG-4 is divided into subgroups HG- I , HG- II , and HG-III by rDNA-ITS sequence analysis [10] and analysis of fatty acids [11]. The authors identified *R. solani* AG-4 causing basal stem rot of broccoli based on the morphological characteristics and anastomosis test. It is considered that subgroups of *R. solani* AG-4 isolates identified in this study could be classified by the aforementioned methods via further study.

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