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Anastomosis Groups and Pathogenicity of *Rhizoctonia solani* Isolates from Radish

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무에서 분리한 *Rhizoctonia solani* 균주들의 균사융합군과 병원성

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ABSTRACT : Incidence of radish disease caused by *Rhizoctonia solani* ranged from 1 to 30% in fields located at Hwaseong, Naju and Yeoncheon in Korea during the growing seasons in 1989, 1990 and 1993. A total of 133 isolates of *R. solani* was obtained from the diseased seedlings, leaves and roots of radish collected. The fungus was most commonly isolated from the roots. Among 133 isolates of *R. solani*, 56 isolates were classified as anastomosis group AG-1 by anastomosis test, 37 isolates as AG-2-1, and 40 isolates as AG-4. Among the isolates of AG-1, 26 isolates were grouped as cultural type IA, and the others as cultural type IB. Cultural types IA and IB of AG-1 were isolated from the leaves, AG-2-1 from the roots, and AG-4 from the seedlings, leaves and roots. Pathogenicity tests revealed that the AG-1(IA) isolates were highly virulent on leaves of radish, but avirulent on the seedlings, petioles and roots. The AG-1(IB) isolates were highly virulent on the leaves, but mildly virulent on the seedlings and avirulent or mildly virulent on the petioles and roots. The AG-2-1 isolates were mildly virulent on the leaves and seedlings and mildly or highly virulent on the petioles and roots. The AG-4 isolates were highly virulent on the seedlings and mildly or highly virulent on the leaves, petioles and roots.

Key words : **Radish, *Rhizoctonia solani*, anastomosis group, pathogenicity.**

Rhizoctonia solani Kühn causes damping-off and root rot of radish (*Raphanus sativus* L.) (1, 2, 6, 9). Infection with the fungus on radish plants results in yield reduction and a decrease in quality of the roots. Lots of the diseased roots have been excluded from the culling after harvest. The disease name of radish caused by *R. solani* was recorded as damping-off in Korea (1). However, root rot caused by the fungus also has commonly occurred in the radish fields.

Various anastomosis groups or cultural types of *R. solani* have been isolated from diseased radish plants (4, 5, 7), and their pathogenicity to radish plants differed (3~5). Kim *et. al.* (7) isolated several anastomosis groups of *R. solani* from diseased seedlings of radish in Korea. However, there have been no studies on anastomosis grouping and pathogenicity of *R. solani* isolates from other parts of radish plants in Korea. In this study, *R. solani* isolates from diseased radish plants were grouped by anastomosis test, and their pathogenicity was investigated. A preliminary report of this study was published (8).

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MATERIALS AND METHODS

Field survey. Radish fields were surveyed at locations Hwaseong, Naju and Yeoncheon in Korea during the growing seasons in 1989, 1990 and 1993. One hundred radish plants in each field were investigated in three replicates.

Isolation and identification. Diseased seedlings, leaves and roots of radish were collected from the

locations surveyed. Nine to 16 mm^2 lesion pieces cut from the diseased radish plants were plated on 2% water agar after surface-sterilizing with 1% sodium hypochlorite solution for 1 min. *Rhizoctonia* sp. was isolated from the lesion pieces after incubation at 25℃ for 1~2 days, and the isolates were transferred to potato dextrose agar (PDA) slants for identification. *R. solani* was identified based on the morphological and cultural characteristics according to the classification of Parmeter and Whitney (12).

Anastomosis test. All isolates of *R. solani* from the diseased radish plants were tested for anastomosis grouping. Each isolate was paired with the standard isolates of AG-1 to AG-7 of *R. solani* obtained from the National Institute of Agro-Environmental Sciences in Japan. Anastomosis test was accomplished by the method indicated by previous workers (10, 11).

Inoculum. Three isolates each of anastomosis groups AG-1(IA), AG-1(IB), AG-2-1 and AG-4 of *R. solani* were used for pathogenicity test to radish plants. Each isolate was cultured in PDRC medium (100 ml of potato dextrose broth and 30 g of rice chaff) in a 500 ml-flask at 25~28℃ for 40 days for inoculation.

Pathogenicity test. Radish cultivars Josaengdaehyung, Taebaek and Taeyang were used for pathogenicity test. Inoculation to the seedlings and roots was performed in three replicates in a greenhouse at 18~26℃.

Forty seeds of each cultivar were sown in a plastic pot (16×7×7 cm) with sterile soil in the greenhouse. Some seedlings were removed from the pot 7 days after sowing, and 20 seedlings were cultivated in the pot. Inoculation to the seedlings was made by putting 20 g of each inoculum in the pot 12 days after sowing. The inoculated pots were placed in a chamber with 100% relative humidity at 23~25℃ for 48 hr, then returned to the greenhouse. Virulence rating was made 7 days after inoculation.

Radish seeds were sown in 1/5000a Wagner pots with sterile soil, and one radish plant in each pot was grown in the greenhouse for soil inoculation. Surface soil around the root was digged by a depth of 2.0~2.5 cm 30 days after sowing, and 40 g of each inoculum was placed around the root. The inoculated part was covered with the original soil. The same quantity of PDRC medium was used for the control. The inoculated pots were placed in the greenhouse and sprinkled with water once a day. Virulence rating was determined based on the symptom development on the petioles and roots 22 days after inoculation.

Mycelial disks of 6 mm in diameter obtained from the margins of actively growing cultures of each isolate on PDA were placed on the leaves of radish cultivated for 30 days in Wagner pots. The inoculated pots were placed in the humid chamber like inoculation to seedlings and returned to the greenhouse. Virulence rating was made 5 days after inoculation.

RESULTS

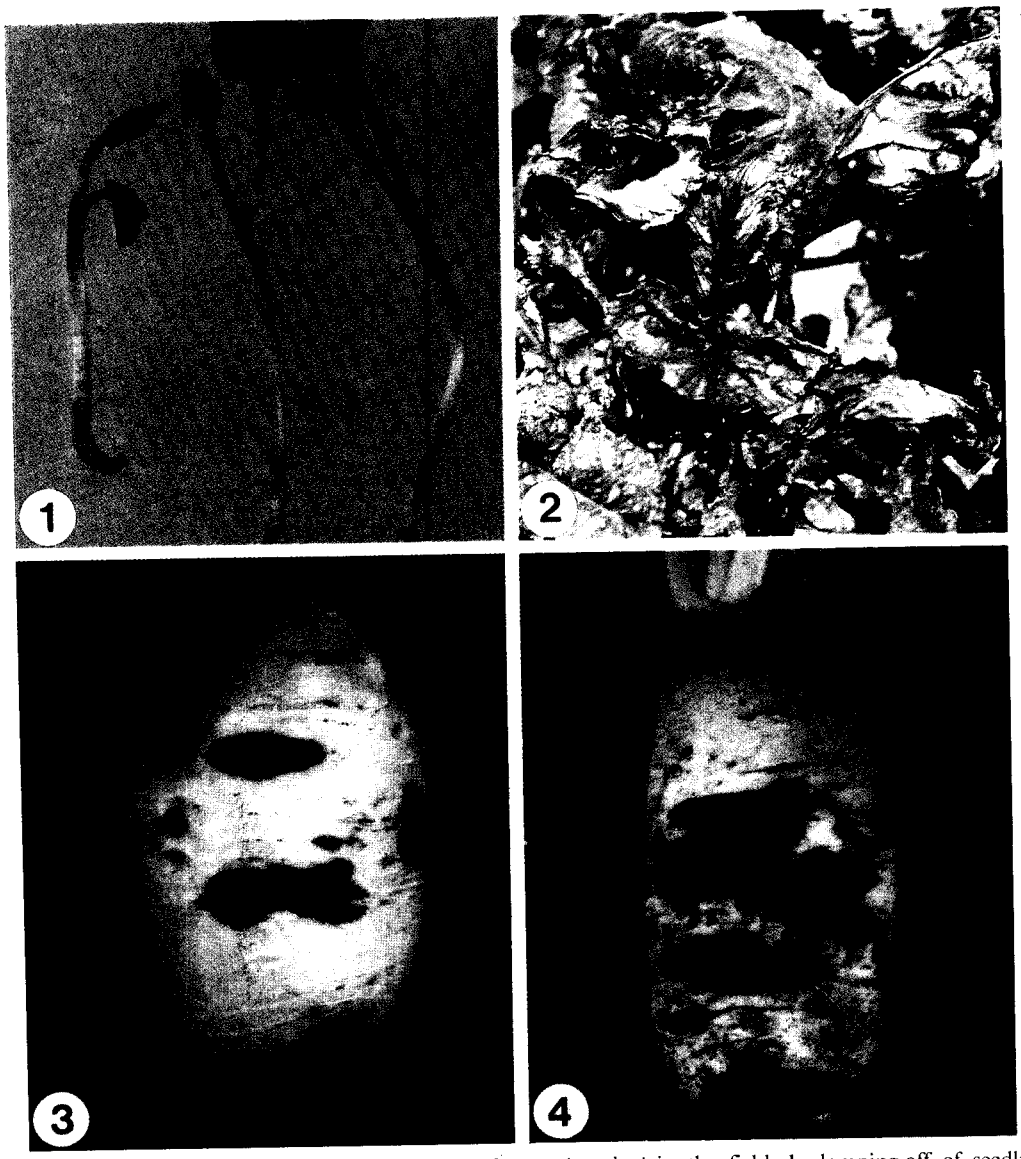
Disease incidence and symptom. Incidence of radish disease caused by *R. solani* ranged from 1 to 30% in fields at three locations during the growing seasons in 1989, 1990 and 1993 (Table 1).

The symptoms on radish plants caused by *R. solani* observed in the field are shown in Fig. 1 through Fig. 4. A symptom appeared as damping-off at the seedling stage (Fig. 1). Infected seedlings died in a few days or weeks. A rot of leaves mostly occurred during the rainy season in summer. The symptom appeared as irregular spots on the leaves, and severely infected leaves blighted and died (Fig. 2). A root rot appeared as brown to dark brown discoloration with cracks like a turtle back at the early

Table 1. Incidence of radish disease caused by *Rhizoctonia solani* in fields at three locations during the investigated time from 1989 to 1993

Location	Survey date		No. of fields	Disease	% infected plants
Hwaseong	August	1990	5[a]	Leaf and root rot	2~30
	October	1993	8	Damping-off and root rot	1~20
Naju	November	1989	6	Root rot	1~12
Yeoncheon	September	1989	3	Root rot	1~ 5

[a]One hundred plants in each field were investigated in three replicates.



Figs. 1~4. Symptoms on radish plants caused by *Rhizoctonia solani* in the field. 1. damping-off of seedlings; 2. leaf rot; 3. root rot at the early stage; 4. root rot at the late stage.

Table 2. Isolation of *Rhizoctonia solani* from diseased seedlings, leaves and roots of radish collected from three locations

Location	No. of isolates			
	Seedling	Leaf	Root	Total
Hwaseong	9	60	15	84
Naju	0	0	37	37
Yeoncheon	0	0	12	12
Total	9	60	52	133

Table 3. Anastomosis groups of *Rhizoctonia solani* isolates from diseased seedlings, leaves and roots of radish

Anastomosis group (cultural type)	No. of isolates			
	Seedling	Leaf	Root	Total
AG-1(IA)	0	26	0	26
AG-1(IB)	0	30	0	30
AG-2-1	0	0	37	37
AG-4	9	4	27	40

stage (Fig. 3). The symptom turned black, enlarged and developed deep cracks and hollows at the late stage (Fig. 4).

Isolation and anastomosis group. A total of 133 isolates of *R. solani* was obtained from the diseased seedlings, leaves and roots of radish collected (Table 2). The fungus was most commonly isolated from the roots.

Among 133 isolates of *R. solani*, 56 isolates were classified as anastomosis group AG-1 by anastomosis test, 37 isolates as AG-2-1, and 40 isolates as AG-4 (Table 3). Among the isolates of AG-1, 26

Table 4. Pathogenicity of *Rhizoctonia solani* isolates belonging to different anastomosis groups by mycelial inoculation to leaves of three radish cultivars

Anastomosis group (cultural type)	Isolate No.	Source isolated	Virulence[a]		
			Josaengdaehyung	Taebaek	Taeyang
AG-1(IA)	900801	Leaf	++[a]	++	++
	900812	Leaf	++	++	++
	900844	Leaf	++	++	++
AG-1(IB)	900802	Leaf	++	++	++
	900820	Leaf	++	++	++
	900833	Leaf	++	++	++
AG-2-1	891157	Root	+	+	+
	891174	Root	+	+	+
	891187	Root	+	+	+
AG-4	900862	Root	++	++	+
	900865	Root	++	++	+
	900867	Root	+	+	+
Control			−	−	−

[a] Virulence was tested using leaves of radish, and the rating was made 5 days after mycelial inoculation. ++ : above 5 cm of average lesion length, + : 2~5 cm of average lesion length, − : no symptom.

Table 5. Pathogenicity of *Rhizoctonia solani* isolates belonging to different anastomosis groups by soil inoculation to radish plants

Anastomsis group (cultural type)	Isolate No.	Virulence								
		Seedling[a]			Petiole[b]			Root[b]		
		Jos.[c]	Taeb.	Taey.	Jos.	Taeb.	Taey.	Jos.	Taeb.	Taey.
AG-1(IA)	900801	−	−	−	−	−	−	−	−	−
	900812	−	−	−	−	−	−	−	−	−
	900844	−	−	−	−	−	−	−	−	−
AG-1(IB)	900802	+	+	+	+	+	+	+	+	+
	900820	+	+	+	−	+	+	−	+	+
	900833	+	+	+	−	+	−	−	+	+
AG-2-1	891157	+	+	+	+	+	++	+	+	+
	891174	+	+	+	+	+	++	++	++	+
	891186	+	+	+	+	++	++	++	+	+
AG-4	900862	++	++	++	+	+	++	++	+	++
	900865	++	++	++	+	++	++	++	++	++
	900867	++	++	++	+	+	+	++	++	+
Control		−	−	−	−	−	−	−	−	−

[a] Virulence rating was made 7 days after inoculation. ++ : most seedlings diseased, + : some seedlings diseased, − : no symptom.

[b] Virulence rating was made 22 days after inoculation, ++ : severe symptom, + : weak symptom, − : no symptom.

[c] Respective abbreviations of cultivars Josaengdaehyung, Taebaek and Taeyang.

isolates were grouped as clutural type IA according to the designation of Watanabe and Matsuda (13), and the others as cultural type IB. Cultural types IA and IB of AG-1 were isolated from the leaves, AG-2-1 from the roots, and AG-4 from the seedlings, leaves and roots.

Pathogenicity. Symptoms of the infected radishes by artificial inoculation with *R. solani* isolates were essentially identical with those observed in the field. Petiole rot was also observed by soil inoculation. Mycelial inoculation tests to radish leaves revealed that the isolates of AG-1(IA) and AG-1(IB) were highly virulent, the AG-2-1 isolates mildly virulent, and the AG-4 isolates mildly or highly virulent (Table 4).

Soil inoculation tests to radish roots revealed that the AG-1(IA) isolates were avirulent on the seedlings, petioles and roots (Table 5). The AG-1(IB) isolates were mildly virulent on the seedlings and avirulent or mildly virulent on the petioles and roots. The AG-2-1 isolates were mildly virulent on the seedlings and mildly or highly virulent on the petioles and roots. The AG-4 isolates were highly virulent on the seedlings and mildly or highly virulent on the petioles and roots.

DISCUSSION

The radish disease caused by *R. solani* appears as a damping-off, root rot or leaf rot. The root rot symptoms most commonly occurs on radish plants. It has been reported that anastomosis groups AG-1 (3), AG-2-1 (3~6), AG-2-2 (4, 5, 14) and AG-4 (3~5) were associated with root rot of radish. The present study revealed that AG-2-1 and AG-4 were mostly associated with the root rot symptoms. It was reported that AG-2-2 was isolated from radish plants in Japan and highly virulent on the roots(4, 5). However, the anastomosis group was not isolated from radish plants in Korea. Imoto *et al.* (5) reported that AG-2-2 was usually isolated from leaves and roots of radish during the summer cultivation. In the present study, *R. solani* was mostly isolated from radish plants during the autumn. Therefore, it is probable that AG-2-2 also causes root rot of radish in Korea during the summer cultivation. It needs further investigation on disease occurrence caused by the anastomosis group on radish plants in Korea.

AG-1, AG-5 and AG-7 of *R. solani* were also isolated from the root rot symptoms of radish but avirulent to weakly virulent on the roots (4). It was reported that cultural type IB of AG-1 was weakly virulent on the roots (5). The authors found that cultural types IA and IB of AG-1 were avirulent to mildly virulent on the roots but highly virulent on the leaves. They were most frequently isolated from the leaves. It is considered that leaf rot is mostly caused by the two cultural types of AG-1. The leaf infection might have originated from soil particles splashed onto the leaves by rain in the field.

Kim *et al.* (7) isolated AG-1 through AG-5 of *R. solani* from diseased seedlings of radish. Imoto *et al.* (5) reported that AG-4 was isolated from damping-off symptoms of radish seedlings but weakly virulent on the seedlings. The authors isolated only AG-4 from the damping-off symptoms, and found that the anastomosis group was highly virulent on the seedlings. It is known that AG-2-1 is highly virulent on the seedlings (5). However, the present study reveals that the anastomosis group is mildly virulent on the seedlings. Accordingly it is likely that damping-off is mostly caused by AG-4 in the fields in Korea. The anastomosis group also attacks all parts of radish plants including the seedlings.

Incidence of radish disease caused by *R. solani* was high as up to 30% in the fields at the three locations in Korea. The disease is very difficult to control because the causal fungus exists in soil. In addition, various anastomosis groups of *R. solani* are associated with the disease occurrence on different parts of radish plants. Kashiwagi (6) reported that there was much difference in occurrence of root rot caused by the fungus among Japanese cultivars of radish. Three Korean cultivars of radish tested by the authors are mostly susceptible to the anastomosis groups. Further study is needed to select resistant radish cultivars.

요 약

1989년, 1990년, 1993년에 국내 화성, 나주, 연천 지역의 포장에서 *Rhizoctonia solani*에 의한 무의 병해발생율은 1~30%였다. 채집한 병든 무의 유묘, 잎, 뿌리에서 총 133균주의 *R. solani*가 분리되었는데, 뿌리에서 가장 일반적으로 분리되었다. *R. solani* 133균주를 균사융합군 검정결과, 56균주는 균사융합군

AG-1, 37균주는 AG-2-1, 40균주는 AG-4로 분류되었다. AG-1의 56균주 중에서 26균주는 배양형 IA로 분류되고, 나머지 균주들은 IB로 분류되었다. AG-1의 배양형 IA와 IB는 모두 잎에서 분리되고, AG-2-1은 뿌리에서, AG-4는 유묘, 잎, 뿌리에서 분리되었다. 병원성 검정결과, AG-1(IA)의 균주들은 무의 잎에는 병원성이 강하였으나, 유묘, 잎자루, 뿌리에는 병원성이 없었다. AG-1(IB)의 균주들은 잎에는 병원성이 강하였으나, 유묘에는 병원성이 약하였으며, 잎자루와 뿌리에는 병원성이 없거나 혹은 약하였다. AG-2-1의 균주들은 잎과 유묘에 병원성이 약하였으며, 잎자루와 뿌리에는 병원성이 약하거나 혹은 강하였다. AG-4의 균주들은 유묘에 병원성이 강하였으며, 잎, 잎자루, 뿌리에는 병원성이 약하거나 혹은 강하였다.

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