

Rhizoctonia solani AG-1(IA)の菌糸融合型の特性と菌株分離場所,分離後年数との関係

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Characteristics in Hyphal Anastomosis of *Rhizoctonia solani* AG-1(IA) in Relation to Locality and Cultural Ages of the Isolates

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Abstract

Twelve isolates of *Rhizoctonia solani* AG-1(IA) from each different location of Korea, four isolates of *R. solani* AG-1(IA) and three isolates of *R. solani* AG-2-2(IIIB) from several locations of Japan having different cultural ages were tested for the reaction and frequency of hyphal anastomosis by WA-cellophane culture. All the isolates except one made cytoplasmic (perfect) fusions without cell death or killing reaction and contact fusions in their self-anastomosis. One AG-2-2(IIIB) isolate made only contact fusions in its self-anastomosis, although it made non-cytoplasmic (imperfect) fusions and contact fusions at low frequency with two different AG-2-2(IIIB) isolates. Of the AG-1(IA) isolates, only the isolate WJB-5 from Korea made cytoplasmic fusions at 57.3% and 54.3% with the isolate 57-6 and HR-1 from Japan, respectively, and the others from Korea made non-cytoplasmic fusions at 47.3-68.0% and contact fusions at 0.7-18.0% with those from Japan. Five AG-1(IA) isolates from Korea including WJB-5 did not make cytoplasmic fusions each other, although non-cytoplasmic fusions and contact fusions occurred among them. The cytoplasmic fusion also did not occur in five pairings of the AG-1(IA) isolates from Japan except the pairing of 57-6 and HR-1. However, the cytoplasmic fusions which occurred between 57-6 and HR-1 occasionally showed abnormal forms of the fused cells, and their frequency was usually lower than that between WJB-5 and 57-6, or WJB-5 and HR-1. Cytoplasmic fusion did not occur in any pairing of three CFIs (cytoplasmic fusion isolates) and ten AG-1(IA) isolates from the same geographic origins of two CFIs, but non-cytoplasmic fusion and contact fusion occurred. The incident property and frequency of cytoplasmic fusion among the CFIs were not varied by subculturing the isolates on PDA. The results revealed that the characteristics in hyphal anastomosis of *R. solani* AG-1(IA) were not related to the locality and cultural ages of the isolates.

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Key words: *Rhizoctonia solani* AG-1(IA), *Rhizoctonia solani* AG-2-2(IIIB), hyphal anastomosis.

INTRODUCTION

Rhizoctonia solani Kühn [*Thanatephorus cucumeris* (Frank) Donk] is a destructive pathogen in many plants and is widely distributed all over the world. Hyphal anastomosis has been used for grouping strains/isolates of *R. solani* since Schultz²²⁾ divided the species into five groups according to the anastomosis^{2,5,15,16,18,19,21,23,28)}. The anastomosis groups of *R. solani* appeared to be genetically segregated^{2,8,16,18)}, and each of them showed a distinctive morphologic and physiologic norm^{16,21,22,24)}. In addition, there were distinct differences in zymogram patterns¹¹⁾, serological groups¹⁾, protein patterns²⁰⁾, and DNA homology⁹⁾ among isolates of the different groups.

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Matsumoto⁹⁾ first found that hyphal fusion occurred between the same strains or certain different strains of *R. solani*. After that, Matsumoto *et al.*¹⁰⁾ reported that there were three types of hyphal fusion of the fungus, namely, perfect, imperfect and contact fusion. It was described that perfect fusion occurred between the closely related strains of *R. solani*, imperfect fusion between less closely related strains, and contact fusion between more distantly related strains^{10,22,26)}. It has been, however, reported that perfect fusion only occurred between the same parent isolates of *R. solani*, imperfect fusion between the isolates from different fields or hosts in the same group, and contact fusion between the different isolates as in the case of imperfect fusion^{4,13,15,16)}. Parmeter *et al.*¹⁸⁾ also reported that both cytoplasmic fusion and cell-wall fusion, followed by cell death, which were considered as perfect fusion and imperfect fusion by them, respectively, occurred in most intra-group pairings of the fungus. On the other hand, clones¹⁷⁾ or anastomosis-compatibility groups¹⁴⁾ which perfectly fused without death of fused cells have been found in the pairings of the isolates within a group of *R. solani* from a same field or different fields.

The processes and the cytological changes in hyphal anastomosis of *R. solani* belonging to group AG-1 and AG-2-2 have been studied by light microscopy or electron microscopy^{13,31-33)}. Many studies also have been accomplished on the characteristics of hyphal anastomosis of basidiospore isolates of *R. solani*^{4,7,12,25,27,29)}, but there have been few studies on those of wild-type isolates in relation to locality and cultural ages. In this study, the characteristics and frequency in hyphal anastomosis of the isolates belonging to group AG-1(IA) of *R. solani* obtained from several locations of Korea, AG-1(IA) and AG-2-2(IIIB) obtained from Japan having different cultural ages were investigated to obtain information on the anastomosis types of rice sheath blight pathogens in nature.

MATERIALS AND METHODS

Isolates used. Sixty isolates of *R. solani* AG-1(IA) from twelve locations of Korea in 1986, were used for the test of reactions of hyphal anastomosis with Japanese standard culture, NIAES 5231 belonging to group AG-1(IA) of *R. solani*. Of them, twelve isolates from each different location of Korea were tested for the reaction and frequency of hyphal anastomosis with four AG-1(IA) isolates and three AG-2-2(IIIB) isolates of *R. solani* from Japan having diverse localities and different cultural ages. In addition, five AG-1(IA) isolates from Weonju in Korea and from Niigata in Japan were used for the more investigation of characteristics of hyphal anastomosis. The isolates from Japan were provided by National Institute of Agro-Environmental Sciences, Saga University, Toyama Agricultural Experiment Station, and Hokuriku National Agricultural Experiment Station in Japan. The locality and isolated year of the isolates used were described in the Tables of experimental results. All the isolates were maintained in PDA (potato dextrose agar) slants for continuous conservation and tests.

Anastomosis test. Hyphal anastomosis was tested by WA-cellophane culture modified from the methods used by previous workers^{16,18)}. The mycelial plugs of 6 mm in diameter from the margins of actively expanding young cultures on PDA were opposed 2-3 cm apart on cellophane resting on 2% WA (water agar) in 9 cm-petri dishes. The cellophane was 6 × 3 cm in size, disinfected in 99.5% ethanol for 8-10 min, washed in sterile distilled water, and dried in sterile filter papers before using. The cultures were incubated at 25C for 24-28 hr until the advancing hyphae from the opposite sides made contact and properly overlapped. Each test was paired in combinations of the isolates used and performed in three replicates.

Stain and observation. The overlapped area of hyphae by WA-cellophane culture was stained with 0.05% toluidin blue O solution and scanned for hyphal anastomosis. Observations were made on the points that hyphae of an isolate contacted with the opposite ones by light microscopy. Frequency of hyphal anastomosis was separately investigated according to the types of anastomosis, which were designated as cytoplasmic (perfect) fusion for showing cytoplasmic connection between the hyphae fused but no death of hyphal cells, non-cytoplasmic (imperfect)

fusion for killing reaction of the hyphal cells fused, and contact fusion for swelling phenomenon of the hyphal tips contacted with the opposite ones without dissolution of the cell wall. Each observation was performed in three replicates with fifty counts.

RESULTS

Reaction and frequency of hyphal anastomosis in relation to locality and cultural ages of isolates

Sixty isolates of *R. solani* AG-1(IA) from twelve locations of Korea made non-cytoplasmic (imperfect) fusions followed by killing reaction and contact fusions with NIAES 5231, Japanese standard culture of *R. solani* AG-1 by anastomosis test.

Twelve isolates of *R. solani* AG-1(IA) from Korea, four isolates of *R. solani* AG-1(IA) and two isolates of *R. solani* AG-2-2(IIIB) from Japan made cytoplasmic (perfect) fusions and contact fusions in the self-anastomosis but not non-cytoplasmic fusions (Table 1). The frequency of cytoplasmic fusions was 49.3–60.7%, and that of contact fusions 3.3–12.7%. The isolate C-127 belonging to group AG-2-2(IIIB) made only contact fusions at 10.0% in its self-anastomosis.

Twelve AG-1(IA) isolates from Korea were tested for hyphal anastomosis in all combinations with four AG-1(IA) isolates and three AG-2-2 (IIIB) isolates from Japan. All the AG-1(IA) isolates from Korea did not make any types of fusion with the AG-2-2(IIIB) isolates from Japan but three types of fusion with the AG-1(IA) isolates (Table 2). Of them, only the isolate WJB-5 made cytoplasmic fusions at 57.3% and 54.0% with the isolate 57-6 and HR-1 from Japan, respectively, and the others made non-cytoplasmic fusions at 47.3–68.0% and contact fusions at

Table 1. Frequency of self-anastomosis of the isolates of *Rhizoctonia solani* AG-1(IA) from Korea, AG-1(IA) and AG-2-2(IIIB) from Japan

Isolate from	Isolate No.	Anastomosis group	Geographic origin	Year isolated	% of fusion		
					Cytoplasmic	Non-cytoplasmic	Contact
Korea	ADB-1	AG-1(IA)	Andong	1986	56.0 ^{a)}	0	5.3
	CCB-5	AG-1(IA)	Chuncheon	1986	58.0	0	10.0
	CJM-3	AG-1(IA)	Cheongju	1986	55.3	0	12.7
	GHM-5	AG-1(IA)	Gimhae	1986	54.0	0	4.7
	GPS-4	AG-1(IA)	Gimpo	1986	56.0	0	3.3
	IRM-8	AG-1(IA)	Iri	1986	56.7	0	4.7
	JJB-1	AG-1(IA)	Jinju	1986	56.7	0	10.7
	NJB-5	AG-1(IA)	Naju	1986	55.3	0	10.7
	NSM-3	AG-1(IA)	Nonsan	1986	50.7	0	11.3
	PTM-5	AG-1(IA)	Pyungtaeg	1986	56.0	0	6.7
	WJB-5	AG-1(IA)	Weonju	1986	57.3	0	8.0
	YCS-1	AG-1(IA)	Youngcheon	1986	60.7	0	8.0
Japan	NIAES 5231	AG-1(IA)	Hukuoka	1976	52.0	0	12.7
	390	AG-1(IA)	Hukuoka	1977	53.3	0	8.0
	57-6	AG-1(IA)	Toyama	1982	60.0	0	8.0
	HR-1	AG-1(IA)	Niigata	1987	49.3	0	12.0
	NIAES 5244	AG-2-2(IIIB)	Hukuoka	1975	60.7	0	12.0
	81-106-1	AG-2-2(IIIB)	Kagoshima	1981	56.0	0	9.3
	C-127	AG-2-2(IIIB)	Hukuoka	1972	0	0	10.0

- a) Observations were made on the points that hyphae of an isolate contacted with the opposite ones of the same isolate under a light microscope after incubating at 25 C for 24–48 hr by WA-cellophane culture. The distance between opposite mycelial mats was 2–3 cm. Average from three replicates with fifty counts of observations.

Table 2. Hyphal anastomosis between the isolates of *Rhizoctonia solani* AG-1(IA) from Korea and those of *R. solani* AG-1(IA) from Japan

Isolate No. from Korea	% of fusion with AG-1(IA) isolates from Japan											
	NIAES 5231			390			57-6			HR-1		
	C ^{a)}	NC	CT	C	NC	CT	C	NC	CT	C	NC	CT
ADB-1	0 ^{b)}	59.3	6.0	0	64.0	8.0	0	50.0	6.7	0	57.3	6.0
CCB-5	0	56.7	6.7	0	59.3	6.7	0	61.3	4.7	0	60.0	6.7
CJM-3	0	47.3	18.0	0	64.0	5.3	0	64.0	4.7	0	57.3	10.7
GHM-5	0	58.7	4.0	0	56.7	7.3	0	54.0	8.7	0	57.3	8.0
GPS-4	0	57.3	9.3	0	64.0	5.3	0	60.7	6.0	0	65.3	8.7
IRM 8	0	64.0	8.0	0	60.0	5.3	0	56.7	8.0	0	60.0	10.0
JJB-1	0	62.7	3.3	0	55.3	10.0	0	62.7	0.7	0	57.3	10.0
NJB-5	0	64.0	8.7	0	61.3	8.7	0	62.7	8.0	0	58.7	4.0
NSM-3	0	59.3	6.0	0	62.7	5.3	0	65.3	6.7	0	50.0	12.0
PTM-5	0	65.3	10.0	0	52.7	8.7	0	50.7	10.7	0	60.0	2.0
WJB-5	0	67.3	5.3	0	62.7	6.7	57.3	0	8.0	54.0	0	6.0
YCS-1	0	61.3	8.7	0	68.0	5.3	0	61.3	7.3	0	66.0	4.7

a) C: cytoplasmic fusion, NC: non-cytoplasmic fusion, CT: contact fusion.

b) Observations were made on the points that hyphae of an isolate contacted with the opposite ones of a different isolate under a light microscope after incubating at 25 C for 24–48 hr by WA-cellophane culture. The distance between opposite mycelial mats was 2–3 cm. Average from three replicates with fifty counts of observations.

0.7–18.0% with all the AG-1(IA) isolates from Japan. The cytoplasmic fusion and the non-cytoplasmic fusion separately occurred between the different AG-1(IA) isolates paired but not together. The contact fusion simultaneously occurred with the cytoplasmic fusion or the non-cytoplasmic fusion.

Five isolates of *R. solani* AG-1(IA) from Korea including WJB-5 were tested for hyphal anastomosis in all possible combinations of them. Non-cytoplasmic fusion and contact fusion occurred between the isolates paired, but cytoplasmic fusion did not occur (Table 3). The frequency of non-cytoplasmic fusions was 49.3–60.0%, and that of contact fusions 4.7–12.0%.

Four isolates of *R. solani* AG-1(IA) and three isolates of *R. solani* AG-2-2(IIIB) from Japan were tested for hyphal anastomosis in all possible combinations of them, respectively. Cytoplasmic fusion occurred only between the isolate 57-6 and HR-1 which made the cytoplasmic fusion with the isolate WJB-5 from Korea in Table 2 (Table 4). In that case, non-cytoplasmic fusion did not occur, but contact fusion occurred at 9.3%. However, the cytoplasmic fusions which occurred between the isolate 57-6 and HR-1 occasionally showed abnormal forms of the fused cells, and their frequency was lower than that between the isolate WJB-5 and 57-6, or WJB-5 and HR-1 shown in Table 2. The other AG-1(IA) isolates paired made non-cytoplasmic fusions and contact fusions, of which frequency was 47.3–60.7% and 6.7–12.0%, respectively, showing that they were almost the same frequency as those of AG-1(IA) isolates from Korea. There were not cytoplasmic fusions among three AG-2-2(IIIB) isolates tested, and the frequency of non-cytoplasmic fusions and contact fusions was 30.7–36.0% and 8.0–13.3%, respectively as shown in Table 4. In that case, the isolate C-127 which made only contact fusions in its self-anastomosis made non-cytoplasmic fusions and contact fusions with two different AG-2-2(IIIB) isolates.

The results revealed that the reaction and frequency of hyphal anastomosis had not any relations in the locality and cultural ages of the isolates.

Features of hyphal anastomosis

The features of hyphal anastomosis which occurred between the tested isolates of *R. solani*

Table 3. Frequency of hyphal anastomosis among the isolates of *Rhizoctonia solani* AG-1(IA) from Korea

Combination of isolates tested	% of fusion		
	Cytoplasmic	Non-cytoplasmic	Contact
ADB-1 + GHM-5	0 ^{a)}	59.3	8.7
ADB-1 + NJB-5	0	59.3	7.3
ADB-1 + NSM-3	0	60.0	4.7
ADB-1 + WJB-5	0	53.3	9.3
GHM-5 + NJB-5	0	52.7	7.3
GHM-5 + NSM-3	0	56.7	8.0
GHM-5 + WJB-5	0	58.0	5.3
NJB-5 + NSM-3	0	56.7	8.7
NJB-5 + WJB-5	0	49.3	12.0
NSM-3 + WJB-5	0	55.3	8.0

a) Average from three replicates with fifty counts of observations by WA-cellophane culture test.

Table 4. Frequency of hyphal anastomosis among the isolates of *Rhizoctonia solani* AG-1(IA) or AG-2-2(IIIB) from Japan

Anastomosis group	Combination of isolates tested	% of fusion		
		Cytoplasmic	Non-cytoplasmic	Contact
AG-1(IA)	NIAES 5231 + 390	0 ^{a)}	60.7	12.0
	NIAES 5231 + 57-6	0	56.7	7.3
	NIAES 5231 + HR-1	0	58.0	6.7
	390 + 57-6	0	47.3	6.7
	390 + HR-1	0	54.7	11.3
	57-6 + HR-1	30.7 ^{b)}	0	9.3
AG-2-2(IIIB)	NIAES 5244 + 81-106-1	0	30.7	12.0
	NIAES 5244 + C-127	0	34.7	13.3
	81-106-1 + C-127	0	36.0	8.0

a) Average from three replicates with fifty counts of observations by WA-cellophane culture test.

b) The cytoplasmic fusions occasionally showed abnormal forms of the fused cells.

AG-1(IA) were shown in Plate I. In cytoplasmic fusion, the cytoplasmic connection without cell death or killing reaction occurred between the hyphae fused (Plate I, A and B). Occasionally attracting phenomenon was observed in the form of hyphal curve from one to another or each other in the cytoplasmic fusion. The fused points of hyphae stained with 0.05% toluidin blue O solution were dyed slightly reddish or normal color in the cytoplasmic fusion. The normal color of hyphae stained with the solution was reddish brown or reddish blue.

Contact fusion which occurred in self-anastomosis or between different isolates showed the swelling phenomenon without dissolution of cell walls of hyphal tips contacted with the opposite hyphae (Plate I, C and D). Hyphal tip cells contacted were normally stained with 0.05% toluidin blue O solution in the case of contact fusion prior to the occurrence of cytoplasmic fusion, but they were dyed deep blue in the case of that of non-cytoplasmic fusion. Especially both hyphal tip cell and the opposite hyphal cell which were contacted with each other appeared to deep blue in the case of contact fusion resulting in non-cytoplasmic fusion with killing reaction (Plate I, E).

In the non-cytoplasmic fusion, the so-called killing reaction was characterized by the death of hyphal cells fused (Plate I, F and G). In that case, cytoplasmic connection did not occur between the hyphal cells fused because of no dissolution of the plasma membrane. The fused cells and occasionally up to one or two adjacent cells of each side hypha were dyed deep blue by

staining with 0.05% toluidin blue O solution. Sometimes attracting phenomenon also occurred in the non-cytoplasmic fusion as the same form shown in the cytoplasmic fusion (Plate I, G).

Any types of fusion were not observed between the AG-1(IA) isolate and the AG-2-2(IIIB) isolate of *R. solani* (Plate I, H). In that case, both side hyphae of two isolates opposed independently grew each other and were normally stained with the staining solution.

Characteristics in hyphal anastomosis of CFIs (cytoplasmic fusion isolates)

The isolates which made cytoplasmic fusions each other in anastomosis tests were designated as CFIs. Three CFIs of *R. solani* AG-1(IA) were tested for the characteristics of hyphal anastomosis with ten different isolates of *R. solani* AG-1(IA) from Weonju in Korea and Niigata in Japan which were isolated from the same places as two CFIs. The CFIs made non-cytoplasmic fusions and contact fusions with all the isolates from the same geographic origins of two CFIs but not cytoplasmic fusions (Table 5). The frequency of non-cytoplasmic fusions and contact fusions was 48.7–68.0% and 6.0–17.3%, respectively, which were similar to those of general non-cytoplasmic fusions and contact fusions shown in Table 2, 3 and 4. The result showed that the incident property of cytoplasmic fusions had not any relations to the locality of the isolates.

Three CFIs of *R. solani* AG-1(IA) were tested by pairing in all combinations of them to ascertain consistency of the cytoplasmic fusion through five subcultures on PDA. The incident property of cytoplasmic fusions among the CFIs was not varied by subculturing the isolates, and the tendency of frequencies among them was consistent (Table 6).

Table 5. Hyphal anastomosis between three CFIs (cytoplasmic fusion isolates) of *Rhizoctonia solani* AG-1(IA) and the different isolates from the same geographic origins of two CFIs

Geographic origin	Isolate No. a)	Year isolated	% of fusion with CFIs								
			WJB-5			HR-1			57-6		
			C b)	NC	CT	C	NC	CT	C	NC	CT
Weonju in Korea	WJB-1	1986	0 c)	62.7	12.0	0	58.0	8.7	0	64.0	10.0
	WJB-2	1986	0	64.7	12.7	0	60.7	6.7	0	49.3	16.7
	WJM-3	1986	0	67.3	10.7	0	62.7	11.3	0	65.3	8.0
	WJM-7	1986	0	63.3	13.3	0	52.0	14.0	0	62.7	10.7
	WJS-2	1986	0	58.7	16.0	0	60.7	10.7	0	59.3	9.3
Niigata in Japan	HR-2	1987	0	54.7	8.0	0	54.0	10.7	0	59.3	10.0
	HR-3	1987	0	68.0	11.3	0	60.7	11.3	0	60.7	13.3
	HR-4	1987	0	56.7	9.3	0	50.7	7.3	0	51.3	17.3
	HR-5	1987	0	48.7	6.0	0	60.0	9.3	0	63.3	14.7
	HR-6	1987	0	57.3	6.0	0	56.0	10.7	0	62.7	12.7

a) The isolates used were classified as group AG-1(IA) by hyphal anastomosis with standard cultures.

b) C: cytoplasmic fusion, NC: non-cytoplasmic fusion, CT: contact fusion.

c) Average from three replicates with fifty counts of observations by WA-cellophane culture test.

Table 6. Hyphal anastomosis among CFIs (cytoplasmic fusion isolates) of *Rhizoctonia solani* AG-1(IA) through subcultures on PDA

Combination of CFIs tested	Frequency of cytoplasmic fusions tested through five subcultures				
	1	2	3	4	5
WJB-5+57-6	++ a)	++	++	++	++
WJB-5+HR-1	++	++	++	++	++
57-6+HR-1	+	+	+	+	+

a) The interval for subcultures was 30–40 days. Result of three separate tests. ++: above 40% of cytoplasmic fusions, +: below 40% of cytoplasmic fusions. The cytoplasmic fusions which occurred between 57-6 and HR-1 occasionally showed abnormal forms of the fused cells.

DISCUSSION

Three types of fusion have been reported in hyphal anastomosis between strains or isolates within a group of *R. solani*^{10,13,15,16,18,22,26}. They have been described as perfect fusion or cytoplasmic fusion, imperfect fusion or cell-wall fusion, and contact fusion. In this study, the types of hyphal fusion were divided into cytoplasmic (perfect) fusion, non-cytoplasmic (imperfect) fusion and contact fusion. The color of fused hyphal cells or fused points were differently observed according to the type of fusion by staining with 0.05% toluidin blue O solution. Especially the fused cells and occasionally up to one or two adjacent cells of each hypha fused appeared to deep blue in the case of non-cytoplasmic fusion by staining with the solution. The deep blue color implied the death of fused hyphal cells or the killing reaction showing the granulation and vacuolation which were described by previous workers^{13,18,31}.

It has been reported that cytoplasmic fusion without killing reaction only occurred between the same parent isolates of *R. solani*^{4,13,15,16,18}. On the other hand, Ogoshi and Ui¹⁷ found the isolates of *R. solani* belonging to the same clone in which cytoplasmic fusion without death of fused cells occurred. They also observed that there were many clones of the fungus in one field, and that the distribution of one clone was limited within only small area in the field. Recently Nishimura and Sugimoto¹⁴ reported that there was the same anastomosis-compatibility (a-c) group in which cytoplasmic fusion occurred, and there were many a-c groups of *R. solani* AG-2-2 in a field but very few between different fields. The authors found that the cytoplasmic fusion without killing reaction occurred between the isolates of *R. solani* AG-1(IA) from different locations as well as between the same parent isolates. In that case, non-cytoplasmic fusion did not occur, but contact fusion occurred. Occasionally abnormal forms of the fused cells were observed in the cytoplasmic fusion which occurred between the isolate 57-6 and HR-1. This phenomenon was similar to the 50% S reaction observed by Flentje and Stretton⁴ or the intermediate type of perfect fusion and cell-death fusion by Nishimura and Sugimoto¹⁴. It requires more studies on the factor and the mechanism of the phenomenon.

The result in this study revealed that cytoplasmic fusion and non-cytoplasmic fusion separately occurred between different isolates of *R. solani* AG-1(IA) but not together. Parmeter *et al.*¹⁸ reported that both cytoplasmic fusion and cell-wall fusion followed by cell death occurred in most intra-group pairings of *R. solani*. However, the cytoplasmic fusion described by them was the same as the imperfect fusion with killing reaction reported by other workers^{10,13,15,16,22}. Therefore, it corresponded to the non-cytoplasmic fusion described by authors. It has been reported that contact fusion occurred between different isolates within a group^{15,16,18}. The result by authors, however, showed that contact fusions usually occurred between the isolates paired in the cytoplasmic fusion as well as in the non-cytoplasmic fusion.

In this study, one AG-2-2(IIIB) isolate of *R. solani* made only contact fusions in its self-anastomosis. However, the isolate made non-cytoplasmic fusions and contact fusions at low frequency with two different AG-2-2(IIIB) isolates. Kernkamp *et al.*⁹ showed that one variant failed to fuse with all other variants of the same parent isolate. Kuninaga⁷ reported that there were some field isolates of *R. solani* AG-2-2 with little or no self-anastomosis. Yokoyama *et al.*³⁰ also ascertained non-self-anastomosis isolates from fields, and found that the frequency of fusions between non-self-anastomosis isolates and normal isolates was low. It has not yet been known about the genetic factors controlling the success or failure of fusions in the self-anastomosis.

Field isolates of *R. solani* were usually heterokaryotic⁴, and the heterokaryon formation was controlled by two linked genes³. The heterokaryons were synthesized through hyphal anastomosis of single-basidiospore isolates from one parent or some lines^{12,27,29}. Stretton *et al.*²⁵ found that completely successful anastomosis occurred between a homokaryotic parent and its progeny or between the progeny, and killing reaction between closely related isolates from other single basidiospore. The killing reaction was a somatic or vegetative incompatibility reaction indicat-

ing a heterogenic incompatibility of the isolates²⁾.

The results by authors revealed that the incident property of cytoplasmic fusion and non-cytoplasmic fusion had no particular relations to the locality and cultural ages of the isolates, indicating that there were some genetical differences between CFIs (cytoplasmic fusion isolates) and the other isolates in group AG-1(IA). Recently, it is said that the occurrence and damage of rice sheath blight by *R. solani* are becoming serious problems in the south-eastern Asian countries. Therefore, further study to solve the differences in genetical level of *R. solani* will give a clue to establish strategies for control measures. The data suggest that the CFIs of *R. solani* AG-1(IA) constitute a somatic compatibility of hyphal anastomosis in nature.

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和 文 摘 要

金 完主・吉野嶺一: *Rhizoctonia solani* AG-1 (IA) の菌糸融合型の特性と菌株分離場所, 分離後年数との関係

分離場所の異なる韓国産 *Rhizoctonia solani* AG-1 (IA) 12 菌株と分離場所および分離後年数の異なる日本産 *R. solani* AG-1 (IA) 4 菌株, AG-2-2 (IIIB) 3 菌株との間の菌糸融合型とその頻度を調査した。自己菌糸融合では AG-2-2 に属する 1 菌株が接触融合のみを起こしたが, 他の供試菌株では細胞質融合と接触融合の両者が認められた。韓国産菌株のうち, WJB-5 (IA) は日本産 AG-1 (IA) 菌株の 57-6 および HR-1 と細胞質融合を起こし, その頻度はそれぞれ 57.3% と 54.3% であった。その他の韓国産菌株は日本産 AG-1 (IA) 菌株と非細胞質融合と接触融合のみを起こした。WJB-5 を含む韓国産 AG-1 菌株間の菌糸融合でも非細胞質融合と接触融合のみが認められた。また, 日本産 AG-1 菌株間の菌糸融合でも, 57-6 と HR-1 の組合せを除いては細胞質融合は認められず, 57-6 と HR-1 菌株間の細胞質融合においても融合細胞の形態が異常となる場合があり, 融合頻度も WJB-5 と 57-6 あるいは WJB-5 と HR-1 間の頻度より低かった。細胞質融合を起こす上記 3 菌株のうち 2 菌株と同一地点から分離された AG-1 (IA) 菌 10 菌株について相互に菌糸融合を試みたが, いずれの組合せにおいても細胞質融合は認められなかった。細胞質融合菌株間の融合特性およびその頻度は PDA 培地上での継代培養によっても変化しなかった。以上の結果から, *R. solani* AG-1 (IA) の菌糸融合特性は菌株の分離場所および分離後年数とは無関係であるものと考えられる。

Explanation of plate

Plate I Reactions in hyphal anastomosis of the isolates of *Rhizoctonia solani* AG-1(IA). The letter and number in each figure indicate the isolates tested. The longer arrows show the direction of hyphal growth, and the shorter one the point of anastomosis. Each figure was magnified $\times 300$.

- A. Cytoplasmic fusion between different isolates in the same group AG-1(IA).
- B. Cytoplasmic fusion between different isolates in the same group AG-1(IA) showing attracting phenomenon.
- C. Contact fusion prior to occurrence of cytoplasmic fusion in self-anastomosis.
- D. Contact fusion prior to occurrence of non-cytoplasmic fusion.
- E. Contact fusion resulting in non-cytoplasmic fusion with killing reaction.
- F. Non-cytoplasmic fusion showing killing reaction.
- G. Non-cytoplasmic fusion showing killing reaction after attracting phenomenon, and contact fusion resulting in non-cytoplasmic fusion.
- H. No fusion between the AG-1(IA) isolate (CJM-3) and the AG-2-2(IIIB) isolate (81-106-1).

Plate I

