THE KOREAN JOURNAL OF MYCOLOGY
Vol. 21, No 3, p188-194 September 1993

Printed in S. KOREA

Anastomosis Types and Hyphal Interactions among Different Location and Field Isolates of *Rhizoctonia solani* AG-1(IA), *R. oryzae* and *R. oryzae-sativae*

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Rhizoctonia solani AG-1(IA), *R. oryzae*, *R. oryzae-sativae*의 다른 地域 및 圃場分離 菌株들간의 菌絲融合型과 菌絲相互作用

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ABSTRACT: Anastomosis types and hyphal interactions in culture among different location and field isolates of *Rhizoctonia solani* AG-1(IA), *R. oryzae* and *R. oryzae-sativae* were examined. In the pairings of *R. solani* AG-1(IA) isolates, cytoplasmic fusion only occurred in the self-anastomoses, and non-cytoplasmic fusion occurred in the other combinations. In the pairings of *R. oryzae* isolates, cytoplasmic fusion occurred in six combinations between different location isolates and in two combinations between different field isolates from the same locations as well as in the self-anastomoses. In that case, four isolates of the fungus reciprocally made the cytoplasmic fusion. In the pairings of *R. oryzae-sativae* isolates, only non-cytoplasmic fusion occurred among the different location and field isolates, in which cytoplasmic fusion also occurred in the self-anastomoses. When non-cytoplasmic fusion isolates(NCFIs) of *R. solani* AG-1(IA) were opposed on PDA, a killing zone developed between the NCFIs paired after incubation. The killing zone also developed between the NCFIs of *R. oryzae* paired. No killing zone developed between the cytoplasmic fusion isolates(CFIs) of *R. oryzae*, in which mycelia of the CFIs intermingled with each other without formation of any demarcation line. An entangled zone instead of the killing zone developed between the NCFIs of *R. oryzae-sativae*.

KEYWORDS: Anastomosis type, *Rhizoctonia* spp., Killing zone.

Rhizoctonia solani Kühn has a wide host range and a saprophytic character in soil. Hyphal anastomosis has been used for grouping isolates of the fungus(Ogoshi, 1976; Parmeter *et al.*, 1969; Richter and Schneider, 1953; Schultz, 1937). Matsumoto *et al.*(1932) first reported three types of hyphal anastomosis of *R. solani* as perfect, imperfect and contact fusion. Perfect fusion occurred between the same parent isolates, and imperfect fusion between isolates belonging to the same anastomosis group derived from different fields or hosts(Flentje and Stretton, 1964; Nishimura and Sugimoto, 1983; Ogoshi, 1976). It has been reported that the group of *R. solani* isolates which reciprocally makes perfect fusion is limited within small area in the field(Nishimura and Sugimoto, 1985; Ogoshi and Ui, 1983). However, Kim and Yoshino(1988) found that cytoplasmic(perfect) fusion rarely occurred between the isolates of *R. solani* AG-1(IA) from different geographic origins. Kim *et al.*(1989) also described three types of hyphal anastomosis, namely, cytoplasmic, non-cytoplasmic and semi-cytoplasmic fusion. Anastomosis types of *Sclerotium fumigatum* Nakata [synonym

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of *Rhizoctonia fumigata* (Nakata *ex* Hara) Gunnell and Webster] and *Rhizoctonia oryzae-sativae* (Sawada) Mordue also were described(Inagaki and Naiki, 1987). In the present study, anastomosis types and hyphal interactions in culture among different location and field isolates of *R. solani*, AG-1(IA) *R. oryzae* Ryker *et* Gooch and *R. oryzae-sativae* were examined.

Materials and Methods

Isolates : Sixteen isolates each of *R. solani* AG-1(IA), *R. oryzae* and *R. oryzae-sativae* were used for the study on anastomosis types occurring among the isolates. The isolates were obtained from diseased rice plants collected from different locations and fields in Korea.

Anastomosis test : The isolates of each species were paired in all possible combinations of them. Hyphal anastomosis was tested by water agar (WA)-culture method used by previous workers (Ogoshi, 1976; Parmeter *et al.*, 1969). 6 mm-diameter mycelial disks obtained from the margins of actively growing cultures on potato dextrose agar (PDA) were opposed 2-3 cm apart on 2% water agar in 9 cm-diameter Petri dishes. The plates were incubated at 25℃ for 24-48 hr until the advancing hyphae from the opposite sides made contact each other and properly overlapped. The overlapped area of hyphae by WA-culture method was stained with 0.05% toluidin blue 0 solution and scanned for hyphal anastomosis. Anastomosis types were classified into cytoplasmic, non-cytoplasmic and semi-cytoplasmic fusion according to the designation by Kim *et al.*(1989).

Culture test of hyphal interaction : The isolates of each species were paired each other. 6 mm-diameter mycelial disks obtained from the margins of actively growing young cultures on PDA were opposed 3.0-3.5 cm apart on PDA in 9 cm-diameter Petri dishes. The plates were incubated at 25℃ in the dark. Observations were made after 4 days of incubation.

Results

In the pairings of *R. solani* AG-1(IA) isolates, cytoplasmic fusion only occurred in the self-anastomoses, and non-cytoplasmic fusion occurred in the other combinations(Table 1).

In the pairings of *R. oryzae* isolates, cytoplasmic fusion occurred in six combinations between different location isolates and in two combinations between different field isolates from the same locations as well as in the self-anastomoses(Table 2). In that case, four isolates of the fungus reciprocally made the cytoplasmic fusion.

In the pairings of *R. oryzae-sativae* isolates, only non-cytoplasmic fusion occurred among the different location and field isolates, in which cytoplasmic fusion also occurred in the self-anastomoses (Table 3).

Microscopic observations of the anastomosis types between the isolates of each *Rhizoctonia* species are shown in Fig. 1 through Fig. 4. In the non-cytoplasmic fusion of *R. solani* AG-1(IA), *R. oryzae* and *R. oryzae-sativae*, a killing reaction was observed, which was characterized by death of fused hyphal cells(Fig. 1, 2 and 4). In the cytoplasmic fusion of *R. oryzae*, cytoplasmic connection without cell death or killing reaction occurred between the hyphae fused(Fig. 3).

When non-cytoplasmic fusion isolates(NCFIs) of *R. solani* AG-1(IA) were opposed on PDA, a killing zone developed between the NCFIs paired after incubation(Fig. 5). The killing zone also developed between the NCFIs of *R. oryzae* paired(Fig. 6). No killing zone developed between the cytoplasmic fusion isolates(CFIs) of *R. oryzae*(Fig. 7), in which mycelia of the CFIs intermingled with each other without formation of any demarcation line. No killing zone developed between the NCFIs of *R. oryzae-sativae* paired(Fig. 8). In that case, an entangled zone replaced the killing zone.

Discussion

It has been reported that perfect fusion occurs between closely related strains of *R. solani*, imperfect fusion between less closely related strains, and contact fusion between more distantly related

Table 1. Hyphal anastomosis among field isolates of *Rhizoctonia solani* AG-1(IA) from different locations

Series No.	Isolate tested	Location	Anastomosis type between the isolates paired														
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	RSCC6-3	Chuncheon	C[a]	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2	RSCC7-4	Chuncheon		C	N	N	N	N	N	N	N	N	N	N	N	N	N	N
3	RSCW6-3	Cheolwon			C	N	N	N	N	N	N	N	N	N	N	N	N	N
4	RSCW10-2	Cheolwon				C	N	N	N	N	N	N	N	N	N	N	N	N
5	RSGC5-4	Gimcheon					C	N	N	N	N	N	N	N	N	N	N	N
6	RSGC9-2	Gimcheon						C	N	N	N	N	N	N	N	N	N	N
7	RSIR1-1	Iri							C	N	N	N	N	N	N	N	N	N
8	RSIR3-3	Iri								C	N	N	N	N	N	N	N	N
9	RSJY6-2	Jinyang									C	N	N	N	N	N	N	N
10	RSJY9-1	Jinyang										C	N	N	N	N	N	N
11	RSMY2-4	Milyang											C	N	N	N	N	N
12	RSMY3-4	Milyang												C	N	N	N	N
13	RSNY3-1	Naju													C	N	N	N
14	RSNY7-3	Naju														C	N	N
15	RSSW5-5	Suwon															C	N
16	RSSW6-2	Suwon																C

[a] C : cytoplasmic fusion, N : non-cytoplasmic fusion.

Table 2. Hyphal anastomosis among field isolates of *Rhizoctonia oryzae* from different locations

Series No.	Isolate tested	Location	Anastomosis type between the isolates paired															
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	ROCC1-1	Chuncheon	C[a]	N	N	N	N	N	N	N	N	N	N	N	N	C	N	N
2	ROCC9-2	Chuncheon		C	N	C	N	N	C	C	N	N	N	N	N	N	N	N
3	ROCW1-3	Cheolwon			C	N	N	N	N	N	N	N	N	N	N	N	N	N
4	ROCW5-2	Cheolwon				C	N	N	C	C	N	N	N	N	N	N	N	N
5	ROGC5-1	Gimcheon					C	C	N	N	N	N	N	N	N	N	N	N
6	ROGC10-2	Gimcheon						C	N	N	N	N	N	N	N	N	N	N
7	ROIR8-4	Iri							C	C	N	N	N	N	N	N	N	N
8	ROIR10-3	Iri								C	N	N	N	N	N	N	N	N
9	ROJY3-2	Jinyang									C	N	N	N	N	N	N	N
10	ROJY8-2	Jinyang										C	N	N	N	N	N	N
11	ROMY2-3	Milyang											C	N	N	N	N	N
12	ROMY9-2	Milyang												C	N	N	N	N
13	RONJ3-5	Naju													C	N	N	N
14	RONJ7-3	Naju														C	N	N
15	ROSW1-2	Suwon															C	N
16	ROSW10-2	Suwon																C

[a] C : cytoplasmic fusion, N : non-cytoplasmic fusion.

Table 3. Hyphal anastomosis among field isolates of *Rhizoctonia oryzae-sativae* from different locations

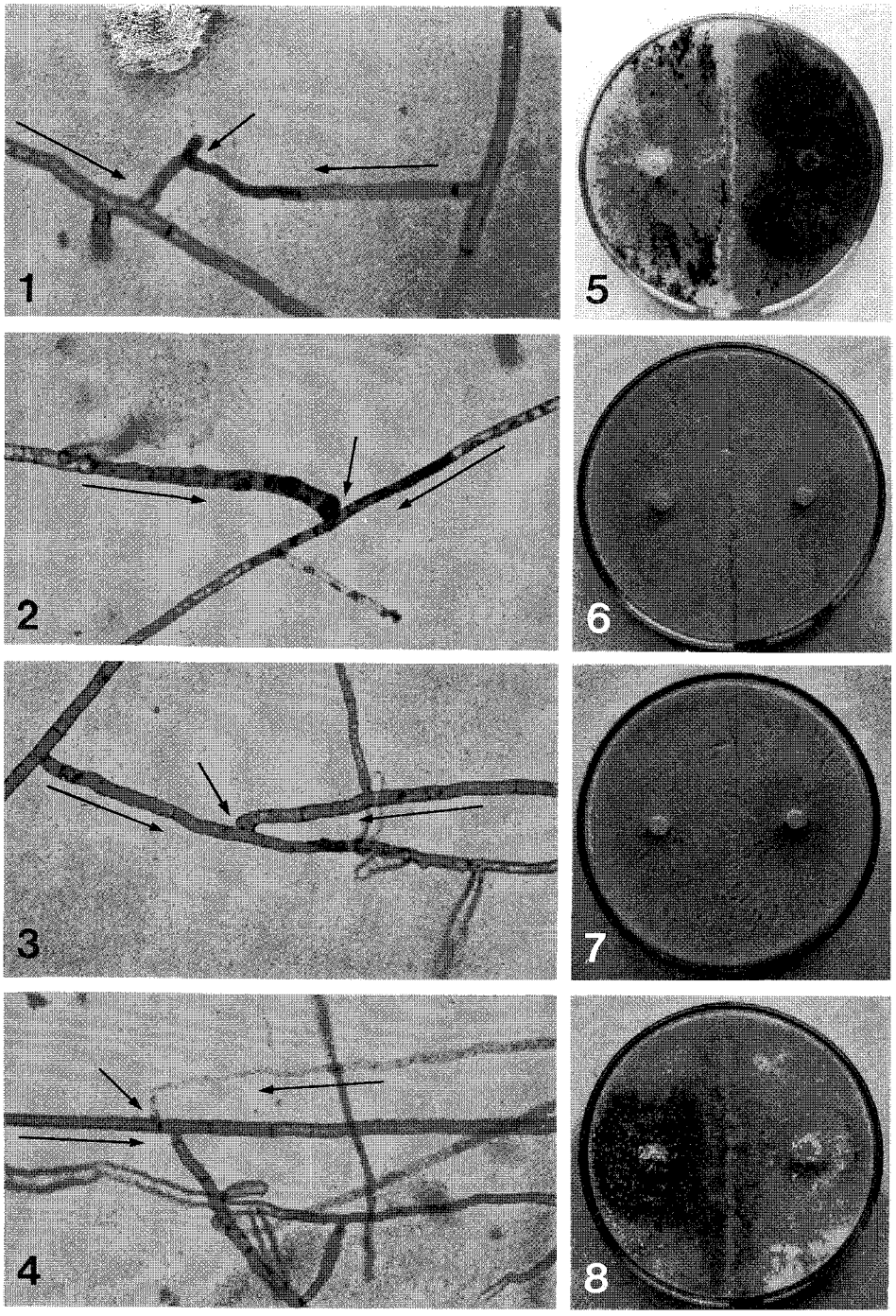
Series No.	Isolate tested	Location	Anastomosis type between the isolates paired														
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	OSCC3-3	Chuncheon	C[a]	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2	OSCC8-2	Chuncheon		C	N	N	N	N	N	N	N	N	N	N	N	N	N	N
3	OSCW3-2	Cheolwon			C	N	N	N	N	N	N	N	N	N	N	N	N	N
4	OSCW7-3	Cheolwon				C	N	N	N	N	N	N	N	N	N	N	N	N
5	OSGC5-2	Gimcheon					C	N	N	N	N	N	N	N	N	N	N	N
6	OSGC10-6	Gimcheon						C	N	N	N	N	N	N	N	N	N	N
7	OSIR5-1	Iri							C	N	N	N	N	N	N	N	N	N
8	OSIR10-2	Iri								C	N	N	N	N	N	N	N	N
9	OSJY2-3	Jinyang									C	N	N	N	N	N	N	N
10	OSJY7-4	Jinyang										C	N	N	N	N	N	N
11	OSMY6-3	Milyang											C	N	N	N	N	N
12	OSMY8-3	Milyang												C	N	N	N	N
13	OSNJ1-2	Naju													C	N	N	N
14	OSNJ5-2	Naju														C	N	N
15	OSSW2-3	Suwon															C	N
16	OSSW10-2	Suwon																C

[a] C : cytoplasmic fusion, N : non-cytoplasmic fusion.

strains(Matsumoto *et al.*, 1932; Schultz, 1937; Tu and Roberts, 1969). In the present study, cytoplasmic(perfect) fusion occurred among some isolates of *R. oryzae* from different locations as shown previously in some isolates of *R. solani* AG-1(IA) by Kim and Yoshino(1988). However, the anastomosis type did not occur among the different location and field isolates of *R. solani* AG-1(IA) and *R. oryzae-sativae*. Semi-cytoplasmic fusion also did not occur among the isolates of each *Rhizoctonia* species examined. Inagaki and Naiki(1987) reported that single perfect fusion group(p-group) of *R. fumigata* and *R. oryzae-sativae* tended to be distributed in a limited part of the field, and p-group isolates were quite similar in the cultural characteristics. The p-group is identical with a clone (Ogoshi and Ui, 1983) or an anastomosis-compatibility(a-c) group of *R. solani*(Nishimura and Sugimoto, 1985). A cytoplasmic fusion(CF) group is also the same as the p-group or a-c group and rarely exists in different field isolates of *Rhizoctonia* species.

Stretton *et al.*(1967) found that completely successful anastomosis occurred between a homokaryotic parent and its progeny or between the progenies, and killing reaction occurred between closely related isolates from other single basidiospores. Thus, it is probable that the isolates belonging to a CF group of *Rhizoctonia* spp. have the same genetic characteristics. The isolates belonging to a non-cytoplasmic(imperfect) fusion(NCF) group of *Rhizoctonia* spp. prevent cytoplasmic exchange each other by the killing reaction. It is considered that the isolates of the NCF group have genetically distinct mycelia as shown in other fungi by previous workers(Adams and Roth, 1967; Punja and Grogan, 1983; Rayner and Todd, 1979).

A killing zone developed between the NCFIs of *R. solani* AG-1(IA) and *R. oryzae* but not between the CFIs. A somatic or vegetative incompatibility has been manifested by formation of a demarcation line between different isolates in other fungi(Adams and Roth, 1967; Esser and Blaich, 1973; Hansen, 1979; Hartl *et al.*, 1975; Ling and



Figs 1-8. Anastomosis types and hyphal interactions between isolates of each *Rhizoctonia* species. The longer arrows in Figs 1-4 show direction of hyphal growth, and the shorter ones point the anastomosis. 1, non-cytoplasmic fusion of *R. solani* AG-1(IA); 2, non-cytoplasmic fusion of *R. oryzae*; 3, cytoplasmic fusion of *R. oryzae*; 4, non-cytoplasmic fusion of *R. oryzae-sativae*; 5, formation of a killing zone between isolates of *R. solani* AG-1(IA); 6, formation of a killing zone between isolates of *R. oryzae*; 7, no formation of killing zone between isolates of *R. oryzae*; 8, formation of an entangled zone between isolates of *R. oryzae-sativae*.

Clark, 1981). The demarcation line has been described as an antagonism zone or a barrage zone, which could be used to distinguish genetically distinct mycelia within the species(Adams and Roth, 1967; Esser and Blaich, 1973; Punja and Grogan, 1983; Rayner and Todd, 1977; Rayner and Todd, 1979). The killing zone observed in the present study might be similar to the antagonism zone by which cytoplasmic exchange is restricted. However, the killing zone is somewhat different from the antagonism zone in terms of generation factors. Rayner and Todd(1977) reported that antagonism zone was due to many factors such as nutrient competition, antibiotic production and toxin removal in interspecific antagonism as well as a killing reaction in intraspecific antagonism. The present study reveals that the killing zone develops in cosequence of a killing reaction in hyphal fusion between isolates of *R. solani* AG-1(IA) and between isolates of *R. oryzae*. Further study is required to clarify difference between the killing zone and antagonism zone.

A killing zone did not develop between the NCFIs of *R. oryzae-sativae*. However, an entangled zone replaced the killing zone. It is suggested that the anastomosis type between the NCFIs of *R. oryzae-sativae* does not result in perfect killing reaction. It needs further study on the formation of the entangled zone between the NCFIs of *R. oryzae-sativae*.

摘 要

Rhizoctonia solani AG-1(IA), *R. oryzae, R. oryzae-sativae*의 다른 地域 및 圃場分離菌株들간의 菌絲融合型과 培養에 의한 菌絲相互作用을 조사하였다. *R. solani* AG-1(IA)菌株들의 相互間 菌絲融合 檢定結果, 細胞質融合은 自己菌絲融合에서만 발생하였으며, 다른 菌株들 相互間에는 非細胞質融合이 발생하였다. *R. oryzae*菌株들의 相互間 菌絲融合 檢定結果, 細胞質融合은 自己菌絲融合에서 뿐 만 아니라 다른 地域分離 菌株들간의 6組合과 같은 地域의 다른 圃場分離 菌株들간의 2組合에서 발생하였다. 이 경우에 이 균의 4菌株 相互間에는 細胞質融合이 발생하였다. *R. oryzae-sativae*菌株들의 相互間 菌絲融合 檢定結果, 細胞質融合은 自己菌絲融合에서만 발생하였으며, 다른 地域 및 圃場分離 菌株들간에는 非細胞質融合만이 발생하였다. *R. solani*의 非細胞質融合菌株들(NCFIs)을 PDA에서 對峙培養한 結果, 致死帶가 형성되었으며, *R. oryzae*의 NCFIs간에도 致死帶가 형성되었다. *R. oryzae*의 細胞質融合菌株들(CFIs)간에는 致死帶가 형성되지 않았는데, 그 CFIs의 菌絲들은 어떤 境界線도 형성하지 않고, 相互間에 混合되었다. *R. oryzae-sativae*의 NCFIs간에는 致死帶가 형성되지 않고, 엉킴대가 형성되었다.

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