DNA Base Sequence Homology in *Rhizoctonia solani* Kühn. VII. Genetic Relatedness between AG-BI and Other Anastomosis Groups

Shiro KUNINAGA* and Ryoza YOKOSAWA*

Abstract

DNA base sequence homology between isolates of AG-BI and other anastomosis groups in *Rhizoctonia solani* was determined by DNA-DNA reassociation kinetics. A comparatively high homology was observed between AG-BI and AG-2. AG-BI isolates showed 36.1 to 48.0 % homology with isolate of AG-2-1 and 34.0 to 46.4% homology with isolates of AG-2-2. While, AG-BI isolates showed 18.7 to 30.2% homology with isolates in AG-3 and AG-6, but consistently low homology (0 to 14.4%) with isolates in the other anastomosis groups. These results suggest that AG-BI and AG-2 should not be considered as separate groups. The phylogenetical relationship between AG-BI and the groups of AG-2, AG-3 and AG-6 is discussed.

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Key Words : *Rhizoctonia solani*, anastomosis groups, DNA homology, genetic relatedness.

Introduction

In a previous paper¹¹⁾, it was demonstrated that each of the anastomosis groups (AGs) in *Rhizoctonia solani* Kühn is a genetically isolated group. This research also suggested that only *R. solani* isolates with similar gene structure successfully anastomose. Also, genetic recombinations through bridging between different AGs were not possible²⁾. However, there is a noteworthy exception to this rule as shown by the recent discovery of a group of "bridging isolates" (AG-BI)¹²⁾. Isolates of AG-BI not only anastomosed with isolates from this group, but also with isolates from AG-2, AG-3 and AG-6. Also AG-BI isolates show greater cultural and physiological similarity with isolates in AG-2, AG-3 and AG-6 than with isolates from other AGs¹³⁾. Thus genetical relationships may exist between AG-BI and these three AGs.

A previous study⁷⁾ provided evidence that AG-BI comprises isolates genetically homogeneous. The purpose of this study was to determine DNA homology between AG-BI and other AGs on the basis of DNA-DNA reassociation kinetics and to elucidate genetical and evolutionary relationships among these AGs of *R. solani*. A preliminary report was published elsewhere¹⁰⁾.

Materials and Methods

Isolate. Three isolates (TE2-4, SHC81 and KA1-1) of *R. solani* selected from AG-

* Department of General Education, Higashi Nippon Gakuen University, Onbetsu, Hokkaido 088-01, Japan 東日本学園大学教養部

BI and twelve isolates from eight other anastomosis groups were used. Origins of these isolates were described previously⁵⁻⁹⁾.

DNA homology. Methods for fungal cultivation, preparation of DNAs, measurement of melting temperature (T_m) of DNAs, shearing of DNAs and determination of DNA fragment size as well as for calculation of DNA homology values were described in previous papers^{4,5)}. DNA-DNA reassociation kinetics were observed in $5\times$ SSC containing 20% DMSO at a temperature about 25 C below T_m .

Results

DNA homology between AG-BI and other anastomosis groups

Table 1 shows DNA homology values between isolates of AG-BI and the three groups (AG-2, AG-3 and AG-6) capable of anastomosis with AG-BI isolates. Comparatively high homology values (18.7 to 48.0%) were observed between AG-BI and AG-2. AG-BI isolates shared 36.1 to 48.0% homology with isolate of the winter crops type (II) in AG-2-1, 34.0 to 43.2% homology with isolate of the rush type (IIIB) in AG-2-2 and 38.9 to 46.4% homology with isolate of the root rot type (IV) in AG-2-2. Homology values between isolates of AG-BI and AG-3 ranged from 23.4 to 30.2%. AG-BI isolates shared 23.7 to 29.1% homology with HG-I isolate in AG-6 and 18.7 to 21.6% homology with GV isolate in AG-6. Fig. 1 and 2 illustrate the profiles of the DNA-DNA reassociation kinetics between DNAs from isolates of AG-BI and these three AGs.

Table 2 shows DNA homology values between isolates of AG-BI and the four groups (AG-1, AG-4, AG-5 and AG-7) not capable of anastomosis with AG-BI isolates. Consistently low homology values (0 to 14.4%) were observed between AG-BI and these AGs. AG-BI isolates shared 0 to 9.2% homology with isolate of the sasakii type (IA) in AG-1 and 1.8 to 14.1% homology with isolate of the web-blight type (IB) in AG-1. AG-BI isolates shared 0 to 6.0% homology with HG-I isolate in AG-4 and 0 to 7.6%

Table 1. DNA homology between isolates of AG-BI and the groups of AG-2, AG-3 and AG-6 in *Rhizoctonia solani*

Isolate	AG-BI			Range
	(TE2-4)	(SHC81)	(KA1-1)	
AG-2-1 ^{a)} , II ^{b)} (R 123)	36.1-47.3 ^{d)}	40.1-48.0	37.2-42.7	36.1-48.0
AG-2-2, IIIB (B 60)	33.9-41.0	34.0-37.3	36.2-43.2	33.9-43.2
AG-2-2, IV (BR-2)	38.9-42.3	39.1-46.0	39.8-46.4	38.9-46.4
AG-3, IV (ST 3-1)	23.9-29.0	24.0-28.7	23.4-30.2	23.4-30.2
AG-6, HG-I ^{c)} (AT2-1)	25.6-29.1	23.7-26.8	24.8-28.8	23.7-29.1
AG-6, GV (NKN 2-1)	19.1-20.6	18.7-21.1	19.8-21.6	18.7-21.6

a) Anastomosis group (Ogoshi, 1976)¹⁶⁾.

b) Cultural type (Watanabe & Matsuda, 1966)²²⁾.

c) Kuninaga & Yokosawa (1984)⁹⁾.

d) The range of homology values is the result of two or three determinations.

Homology values were calculated from the equation by Seidler & Mandel (1971)¹⁸⁾.

Fig. 1. Reassociation kinetics of the mixture of DNA from isolates of AG-BI and AG-2 in *Rhizoctonia solani*. All reactions were carried out in 5×SSC containing 20% DMSO at 62 C (left).

Fig. 2. Reassociation kinetics of the mixture of DNA from isolates of AG-BI and the groups of AG-3 and AG-6 in *Rhizoctonia solani*. All reactions were carried out in 5×SSC containing 20% DMSO at 62 C (right).

Table 2. DNA homology between isolates of AG-BI and the groups of AG-1, AG-4, AG-5 and AG-7 in *Rhizoctonia solani*

Isolate	AG-BI			Range
	(TE2-4)	(SHC81)	(KA1-1)	
AG-1 ^a , IA ^b) (A-10)	0 - 3.1 ^d)	5.9- 9.2	3.1- 8.8	0 - 9.2
AG-1, IB (001-7)	7.0-12.0	1.8- 3.0	7.6-14.1	1.8-14.1
AG-4, HG-I ^c) (Chr-3)	0 - 3.8	4.2- 6.0	1.3- 4.4	0 - 6.0
AG-4, HG-II (UHBC)	0 - 5.6	2.2- 7.6	2.5- 7.4	0 - 7.6
AG-5 (TE1-5)	6.7- 9.3	5.0-13.9	6.2-14.4	5.0-14.4
AG-7 (1535)	5.2- 7.6	0 - 5.8	6.2-14.4	0 -14.4

a), b), d) See footnote in Table 1.

c) Kuninaga & Yokosawa (1984)⁸⁾.

Fig. 3. Reassociation kinetics of the mixture of DNA from isolates of AG-BI and the groups of AG-1, AG-4, AG-5 and AG-7 in *Rhizoctonia solani*. All reactions were carried out in 5×SSC containing 20% DMSO at 62 C.

homology with HG-II isolate in AG-4. Homology values between isolates of AG-BI and AG-5 ranged from 5.0 to 14.4%, and homology between isolates of AG-BI and AG-7 ranged from 0 to 14.4%. Fig. 3 shows the reassociation kinetics between DNAs from isolates of AG-BI and these four AGs.

Discussion

This study demonstrated that AG-BI isolates show comparatively high DNA homology (18.7 to 48.0%) with isolates of AG-2, AG-3 and AG-6 which are capable of anastomosis with AG-BI isolates, whereas markedly low DNA homology (0 to 14.4%) with isolates of AG-1, AG-4, AG-5 and AG-7 which are not capable of anastomosis with AG-BI isolates. These findings suggest that anastomosis behaviour of the "bridging isolates" in AG-BI could provide information on genetical and evolutionary relationships among AGs in *R. solani*.

In a previous paper¹¹⁾, it was stated that isolates of *R. solani* with more than about 30 to 35% homology should be regarded as belonging to the same AG. As judged from this criteria, it is suggested that AG-BI and AG-2, showing 34.0 to 48.0% homology, should not be considered as separate groups. AG-BI isolates also show a relatively high frequency of anastomosis with AG-2 isolates¹³⁾. Such phenomena and the results of DNA homology led to the conclusion that evolutionary changes between isolates in AG-BI and AG-2 still were not completed, and that AG-2 might have phylogenetically originated

from AG-BI. These assumptions are also supported by the observations of cultural and physiological similarity between both groups^{12,13}. It was also reported that within AG-2 two subgroups (AG-2-1 and AG-2-2), showing different frequency of anastomosis, can be distinguished¹¹. Various degrees of anastomosis frequency observed in AG-BI and AG-2 may have played a significant role in their evolution. Whether outbreeding is possible between these groups is a problem yet to be solved in this context.

In this study, consistently low homology (less than 30%) was observed between isolates of AG-BI and other AGs except AG-2. This suggests that AG-BI is genetically isolated from these AGs with the exception of AG-2. On the other hand, AG-BI isolates showed somewhat higher homology (18.7 to 30.2%) with isolates of AG-3 and AG-6 than that with isolates of AG-1, AG-4, AG-5 and AG-7. These results and the observations¹³ that AG-BI isolates can anastomose (but very rarely do) with isolates of AG-3 and AG-6 lead us to speculate on evolutionary close relationships among these AGs. It appears that AG-3 and AG-6 may be populations which have evolved from AG-BI.

Storck & Alexopoulos¹⁹ suggested that the evolution of fungi is usually accompanied by a progressive increase in GC content of DNA. Similar correlation between GC content and evolution was also found among bacteria in the actinomycetes^{3,19} and also among intragenetic variations of yeasts^{15,19,20}. Indeed, AG-BI isolates showed the lowest GC content among all isolates of AGs in *R. solani*⁵. This further supports the assumption that AG-2, AG-3 and AG-6 may have evolved from AG-BI. AG-BI isolates are avirulent¹², whereas isolates of AG-2 and AG-3 exhibit parasitic features^{1,16,17}. This suggests that evolutionary changes of *R. solani* are accompanied by the progressive specialization of parasitic habits. This seems to give a proof of the direction of evolutionary series toward parasitism in plant pathogenic fungi^{14,21,23}. Further studies on genomic analysis of *R. solani* would be useful for elucidating these interesting question.

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和 文 摘 要

国永史朗・横沢菱三：*Rhizoctonia solani* における DNA 塩基配列の相同性 VII. AG-BI と他の菌糸融合群間の遺伝的類縁性

Rhizoctonia solani の菌糸融合群第 BI 群 (AG-BI) と他の菌糸融合群菌株間における遺伝的類縁性を検討するため、DNA-DNA 再会合反応速度解析に基づき DNA 塩基配列の相同性を比較した。AG-BI は AG-2 と比較的高い相同性 (34.0~48.0%) を示し、また AG-3 および AG-6 とでは18.7~30.2%相同性を示したが、その他の菌糸融合群とでは、いずれの場合も著しく低い相同性を示した (0~14.4%)。DNA 相同性比較結果から、AG-BI と AG-2 は相互に遺伝的に近縁なグループであることが示唆され、また AG-BI と AG-3 および AG-6 の菌糸融合群間に系統発生的な関係が認められた。