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Population structure of *Sclerotium rolfsii* in peanut fields

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Sclerotium rolfsii **isolates from peanut fields in Ibaraki were classified into mycelial compatibility groups (MCGs) based on the barrage zone formation. A total of 132 isolates collected from four fields within a 120 m radius in 1994 comprised four MCGs; MCG A (71 isolates), B (34 isolates), C (26 isolates) and D (one isolate). Fields 1 and 2 were occupied exclusively by MCG A. MCG A also predominated in field 3. In field 4, MCGs A, B and C were dominant. Population structure in 3 additional fields was determined in 1997. All 11 isolates from Field 5, which was 400 m distant from field 1, belonged to MCG C. A total of 42 isolates from fields 6 and 7, 2.5 km distant from other fields and 100 m distant from each other, were all MCG A. These results suggested that the population structure of *S. rolfsii* was simple. RAPD fingerprintings showed that most isolates of the same MCG were clonal.**

Key Words——mycelial compatibility; RAPD; *Sclerotium rolfsii*.

Sclerotium rolfsii Sacc. [teleomorph: *Athelia rolfsii* (Curzi) Tu & Kimbrough] is a soil-borne plant pathogenic fungus with a wide host range and worldwide distribution (Punja, 1988). The fungus spreads by mycelial contact with healthy plants and overwinters as sclerotia in soil. Consequently, the disease occurrence is typically endemic, and the spatial distribution of the disease is clustered (Shew et al., 1984).

Nalim et al. (1995) showed that genetic diversity of *S. rolfsii* on peanut was low in Texas; each field contained a small number of mycelial compatibility groups (MCGs), and the same MCGs existed in several geographically distant fields. They also reported that all isolates within an MCG gave an identical ITS-RFLP pattern. The low diversity within *S. rolfsii* isolates from specific geographic regions suggests limited genetic exchange, and in fact, its sexual stage (Punja and Grogan, 1983a) is not commonly observed in nature.

We reported that Japanese isolates of *S. rolfsii* consisted of five ITS-RFLP groups (Okabe et al., 1998). To detail the structure of local geographic populations, we collected 185 isolates from peanut fields in the southern part of Ibaraki. MCGs were genetically characterized by random amplified polymorphic DNA (RAPD) analysis, although they belonged to the same ITS-RFLP group. A brief report of this work was presented previously (Okabe and Matsumoto, 1998).

Material and Methods

Study site Four peanut fields in Kajiuchi, the suburb of Tsukuba, were sampled in August 1994. The fields 1 to 4 were separated by lanes and clustered within a 120 m radius (Fig. 1a). Upland rice, taros and other crops had been grown in rotation with peanut. Ten to 40% of peanut plants in each field were found infected with *S. rolfsii*.

In September 1997, we investigated one additional field in Kajiuchi (field 5) and two in Mamiana (fields 6 and 7) (Figs. 1a, b). Field 5 was on the other side of a road, 400 m northeast from fields 1 to 4. Mamiana was 2.5 km south from Kajiuchi, and the cultivation system was the same as Kajiuchi. Ten to 20% of the plants were infected with *S. rolfsii* in these fields.

Isolate collection Five sclerotia were collected from each diseased plant and disinfested in hypochlorite solution (active chlorine 0.125%) for 5 min before incubation on potato dextrose agar for isolation. Because the isolates from the same plants were always found to be vegetatively compatible and considered to be clonal, one representative isolate from each plant was used in the following tests. A total of 185 representative isolates were obtained.

Mycelial compatibility groups Distinction of MCGs was based on pairing of isolates on Difco oatmeal agar plates at room temperature. When the colonies grew together without a visible border between them, the isolates paired belonged to the same MCGs. However, if the isolates were incompatible, a barrage zone (Punja and Grogan, 1983b) and pigmentation were observed in 2 wk.

RAPD analysis RAPD analysis followed the methods of Punja and Sun (1997) using 24 isolates from 4 MCGs. Mycelia grown in potato-dextrose broth for 1 to 2 wk were used for DNA isolation according to the standard protocol of a DNA isolation kit (Easy-DNA Kit, Invitrogen Corporation, San Diego). Of 40 ten-base primers screened, OPA-09, 15, 16, 19 and OPB-13 (Operon Technologies. Inc., Alameda) distinguished MCGs. PCR reaction mixture of 50 μl contained 5 μl of 10 × Gene *Taq* Universal Buffer (Nippon Gene Co., Tokyo), 400 μM of dNTP mixture (Nippon Gene Co., Tokyo), 10 pM of

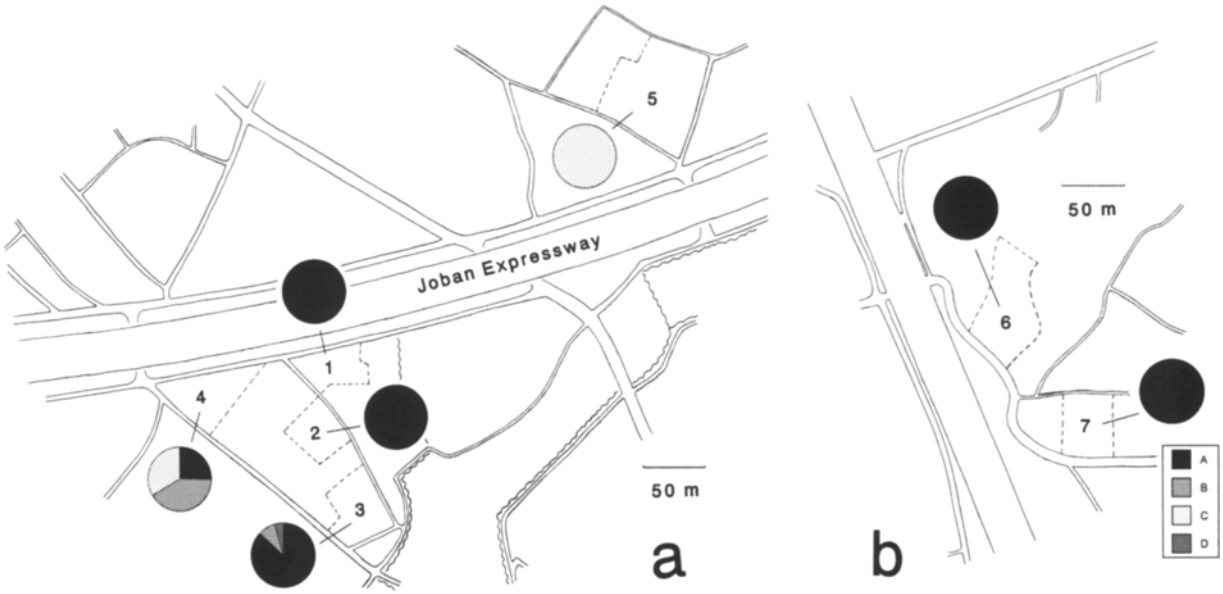

Fig. 1. Distribution of MCGs in fields 1 to 5 (a), and in fields 6 and 7 (b). Pie diagrams summarize frequencies of MCGs. Scale bars: 50 m.

primer, 1 unit of *Taq* DNA polymerase (Nippon Gene Co., Tokyo) and 50 ng of DNA template. PCR amplification consisted of an initial denaturation step at 94°C for 2 min followed by 44 cycles of denaturation at 94°C for 1 min, anealing at 36°C for 1 min, and extension at 72°C for 2 min with a single final extension at 72°C for 5 min. PCR products were subjected to electrophoresis for 60 min at 50 mA on 2% agarose gels (NuSieve 3 : 1, FMC BioProducts, Rockland) in TAE buffer containing ethidium bromide (Sambrook et al., 1989).

Results

Mycelial compatibility groups Eleven, 21, 22, 78, 11, 10 and 32 isolates were obtained from fields 1 to 7, respectively (Table 1). These isolates comprised four MCGs, MCG A (61.1%), B (18.4%), C (20.0%) and D (0.5%). MCG A was found exclusively in fields 1 and 2, and was predominant (86.4%) in field 3. The population in field 4 consisted of MCG A (25.6%), B (41.0%) and C (33.3%). In field 5, all isolates were identified as MCG C. MCG A also occupied fields 6 and 7, which were 2.5 km distant from other fields (Fig. 1). Most diseased plants occured sporadically, and there was only one large patch of diseased plants in field 4. The patch produced 15 isolates, all of which proved to be MCG B.

Table 1. Distribution of MCG among isolates of *Sclerotium rolfsii* collected from infected peanut plants in seven fields in 1994 and 1997.

	MCG A	B	C	D
August 1994				
field 1 (Kajiuchi)	11	0	0	0
2 (Kajiuchi)	21	0	0	0
3 (Kajiuchi)	19	2	0	1
4 (Kajiuchi)	20	32	26	0
September 1997				
field 5 (Kajiuchi)	0	0	11	0
6 (Mamiana)	10	0	0	0
7 (Mamiana)	32	0	0	0

Table 2. RAPD patterns of 24 *Sclerotium rolfsii* isolates.

RAPD type	isolates	MCG
type 1	1–2[a], 2–1, 2–6, 3–3, 3–7[b], 3–12, 4–12, 4–47, 6–2, 6–8, 7–2, 7–25	A
2	1–7[b]	A
3	3–4[b], 3–6, 4–1, 4–10, 4–72	B
4	4–23, 4–28[b], 4–60, 5–3, 5–9	C
5	3–19[b]	D

a) The isolates were designated for the field from which they collected and isolate number within the field. For example, "1–2" referred to isolate 2 from field 1.

b) Isolates 3–7, 1–7, 3–4, 4–28 and 3–19 were referred to as isolates s-54, s-51, s-52, s-53 and s-55 in our previous work (Okabe et al., 1998) and were assigned to ITS-RFLP group 1.

RAPD analysis Three distinct patterns were observed with OPA-19 and two patterns with four other primers (Fig. 2). Combining the results, five RAPD genotypes were recognized among 24 isolates representing four MCGs (Table 2).

Twelve MCG A isolates from 6 fields were assigned to RAPD type 1, and isolate 1–7 (MCG A) was designated as type 2 because of the difference in the OPB-13 pattern. The isolates of MCGs B, C and D belonged to types 3, 4 and 5, respectively.

Discussion

The mycelial compatibility test showed that *S. rolfsii* populations in peanut fields consisted of single or a small number of MCGs. All 15 isolates from one disease locus in field 4 were mycelially compatible, suggesting that the patch had been established by vegetative spread of a single MCG.

MCG A was present in 6 fields within a 1.5 km radius and MCGs B and C in two fields respectively. RAPD analysis showed that the isolates belonging to the same MCGs collected from the different fileds were genetically identical. It has previously been reported that mycelially compatible isolates, which were collected from specific geographic regions, had the same genotypic characters (Smith et al., 1994; Worrall, 1997).

A simple population structure has often been observed in soil-borne plant pathogenic fungi such as *Typhula ishikariensis* (Matsumoto and Tajimi, 1993), *Armillaria ostoyae* (Smith et al., 1994), *Helicobasidium mompa* (Katsumata et al., 1996) and *Sclerotium cepivorum* (Earnshaw and Boland, 1997), suggesting that the dissemination mostly depends on the vegetative reproduction, e.g. sclerotia and mycelial strands. On the other hand, airborne plant pathogenic fungi which spread sexual spores often exhibit great genotypic diversity (Anagnostakis and Kranz, 1987; Kohn et al., 1991; Kohli et al., 1992; Chen and McDonald, 1996).

MCGs A and C were found at different times, i.e., in 1994 and 1997, as was the case with *S. rolfsii* (Nalim et al., 1995) and *T. ishikariensis* (Matsumoto et al., 1996). These observations suggest that MCGs are persistent over time.

Isolate 1–7 differed in RAPD pattern from other MCG A members, lacking one band in OPB-13 pattern. Genetic variation within an MCG have been reported previously with similarly small (Matsumoto et al., 1996; Punja and Sun, 1997). This suggests that minor somatic mutation can occur without affecting mycelial compatibility during the course of clonal growth in the field populations of *S. rolfsii*. However, differences between MCGs also arise

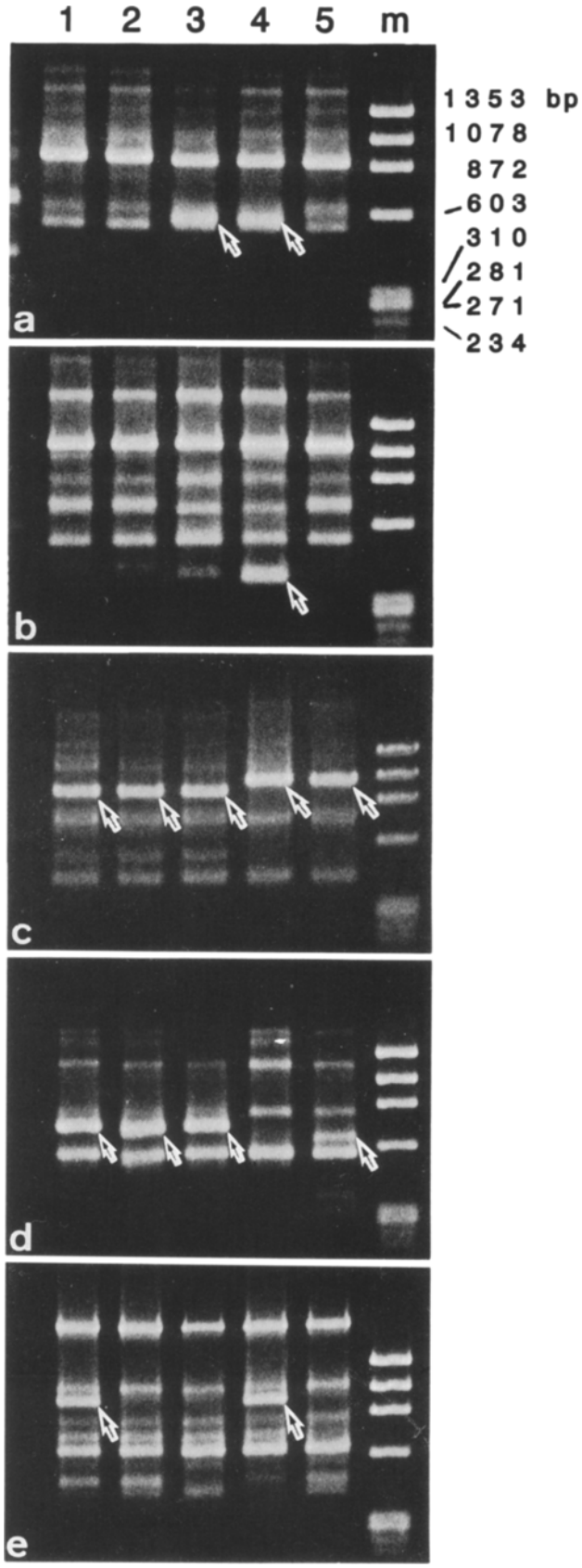

Fig. 2. RAPD patterns generated with primer OPA-09 (a), OPA-15 (b), OPA-16 (c), OPA-19 (d) and OPB-13 (e). Lane 1=isolate 3–7 (type 1), lane 2=isolate 1–7 (type 2), lane 3=isolate 3–4 (type 3), lane 4=isolate 4–28 (type 4), lane 5=isolate 3–19 (type 5), lane m=$\phi\chi$174/*Hae*III. Arrows indicate the amplification products which distinguish the different RAPD types.

from genetic changes that must be larger.

Because asexual reproduction was found to contribute to the population structure in *S. rolfsii* and because major genetic change within local populations are unlikely to occur, it is still unclear how *S. rolfsii* adapts to environmental changes in agricultural ecosystem. Further investigations are needed to reveal the population dynamics and strategy of *S. rolfsii*.

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