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Short Communication

Mycelial compatibility groups of *Sclerotium rolfsii* in South Africa

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***Sclerotium rolfsii*, the causal organism of stem rot or southern blight of groundnut, is widely distributed and has been reported to cause disease in over 500 plant species throughout the world and at least 30 in South Africa. The disease is a problem in most groundnut producing areas in South Africa and no efficient control strategy has yet been developed in this country. Previous studies on the fungus have indicated that diversity within populations of *S. rolfsii* can be defined by placing isolates into mycelial compatibility groups (MCGs). A total of 121 *S. rolfsii* isolates were collected from 15 localities and seven plant species throughout South Africa and paired against each other. Thirteen MCGs were identified, some containing isolates from the same host plant or geographic area, suggesting a possible relationship between MCG and host plant or locality. Other MCGs, however, contained isolates from a variety of hosts from various localities. The population of the fungus in South Africa is diverse, with vague associations between MCG, host and geographical distribution.**

Sclerotium rolfsii Sacc., the causal organism of southern stem rot (SSR) or southern blight of groundnut (*Arachis hypogaea* L.), is widely distributed and was first recorded in South Africa on tobacco (*Nicotiana tabacum* L.) in 1926 (Moore 1926) and on groundnut in 1931 (Doidge and Bottomley 1931). Punja and Grogan (1983a, b) showed that *Sclerotium rolfsii* isolates can be placed into mycelial compatibility groups (MCGs) based on mycelial interactions (Glass and Kuldau 1992) between isolates. According to Kohn *et al*. (1991) mycelial compatibility is an important event in the formation of stable heterokaryons in fungi through vegetative compatibility. The role of MCGs and VCGs (vegetative compatibility groups) is thus important in defining field populations of fungi and facilitating genetic exchange in fungal species, where the teleomorph stage of the life cycle has a minimal impact on the disease cycle (Kohn *et al.* 1991, Leslie 1993).

Population studies of *S. rolfsii* have investigated genetic variation within and between MCGs. Nalim *et al.* first surveyed MCGs in specific fields and in individual plants in Texas in 1995. In their study isolates could be placed into 25 MCGs. DNA amplification patterns resulting from the use of the 18-base oligonucleotide primer NK2, and from restriction digests of the internal transcribed spacer (ITS) region of the rDNA, were examined in a subset of 80 isolates, representing 12 MCGs. Isolates from a single MCG had identical patterns for each marker, and some MCGs shared the same ITS and NK2 patterns. The study further indicated that MCGs having the same DNA patterns were often collected from the same field. Other studies have suggested that MCGs may be associated with either geographical area or host plant (Harlton *et al.* 1995, Punja and Sun 1997). Harlton *et al*. (1995) screened a worldwide collection of *S. rolfsii* isolates and identified 49 MCGs from 119 isolates. Isolates from the same geographical area or host often grouped in the same MCG, but in some cases widely diverse isolates grouped in the same MCG.

Punja and Sun (1997) paired 128 isolates from 36 host species and 23 geographical regions against each other and identified no less than 68 MCGs. No relationship between host of origin and MCG was found, except that many isolates from the same hosts belonged to the same MCG. Conversely, isolates in a specific MCG could have originated from many different hosts. An UPGMA (a cluster analysis by the unweighted paired group method using arithmetic averages) analysis revealed that isolates within the same MCG were genetically diverse, as were isolates from the same geographical area (Punja and Sun 1997). Isolates from the same MCG did, however, group closer together suggesting greater genetic similarity. Isolates from widely distant geographical locations were more distantly related and isolates with identical RAPD patterns grouped in the same MCG and it was suggested that these were probably clonally derived (Punja and Sun 1997).

In a recent study on the genetic structure on MCGs of *S. rolfsii* in South Africa, it was shown for the first time that all

isolates within an MCG can be distinguished from each other using AFLP analysis (Cilliers *et al.* 2000). In the latter study, isolates from two different MCGs were compared using four primer pairs. Additional studies using 10 primer pairs identified polymorphisms between all tested isolates within a specific MCG. Nine different MCGs were also compared using 20 primer pairs and all were found to be genetically dissimilar.

The aim of this study was to investigate the mycological diversity within a larger population of *S. rolfsii* in South Africa and to establish the relationship between MCG, geographical area and host plant. No such study has yet been undertaken on the population of this fungus in South Africa. This study, in combination with the genetic study of the population diversity (Cilliers *et al.* 2000), will increase our understanding of the pathogen which could eventually lead to more effective disease control.

During the 1997/1998, 1998/1999 and the 1999/2000 growth seasons isolates of *S. rolfsii* were collected from host plants and geographical locations as indicated in Table 1. The areas from which the isolates were collected were diverse, representing six of the nine provinces in South Africa. Sclerotia or mycelium was collected from various crop plants as well as other plant species. One to four isolates were collected from each infected plant. Sclerotia or fungal mycelium from infected plants were placed on 50% malt extract agar (MEA) plates and incubated at room temperature (23°C). Single hyphal tips were transferred from germinating sclerotia or growing mycelium to potato dextrose agar (PDA) (Difco Laboratories, Detroit). Cultures were incubated for 10 days at 23°C until numerous sclerotia had formed. All cultures were transferred to PDA slants and stored at 5°C until the MCGs were determined.

Single sclerotia from stored *S. rolfsii* cultures were placed on PDA (100 x 15mm) plates in order to determine the MCG. A system was developed whereby each isolate was paired at least once against itself as a control and against six other isolates. All pairings were conducted twice. Isolates were placed in different MCGs based on the presence of an antagonistic zone, which indicates incompatibility between two paired fungal isolates.

A total of 121 isolates from hyphal tips were collected from 15 localities throughout South Africa (Table 1). Most of the isolates (34) originated from the Potchefstroom area. Between one and thirteen isolates were collected from the other localities (Table 1). Isolates were obtained from the host plants as indicated in Table 1. Pairing of isolates resulted either in the formation of clear barrier zones or the lack thereof (Figure 1). Mycelia of isolates in the same MCG intermingled and developed a white ridge indicating a compatible reaction. Mycelia of isolates from different MCGs formed a clear antagonistic zone in the area of mycelial contact, indicating an incompatible reaction (Figure 1). Isolates could be assigned to one of 13 MCGs which were then numbered from A through M. Replicated pairings produced identical results. Reference isolates are stored at Plant Protection Research Institute, Pretoria and preserved under reference numbers PPRI 7018 to PPRI 7030.

Table 1: Geographic distribution of isolates, host plant, number of isolates per locality, date of collection and number of isolates from each mycelial compatibility group (MCG) of isolates from the population of *Sclerotium rolfsii* in South Africa

Locality	Host plant	Number of isolates	Date of collection	MCG
Potchefstroom	Peanut	13	February 1998	B
		1	March 1998	E
		6	April 1998	4 X B, 2 X E
Potchefstroom	Sunflower	5	February 1998	1 X D, 4 X B
Potchefstroom	Lupin	8	February 1998	7 X C, 1 X B
Lichtenburg	Peanut	7	February 1998	A
Viljoenskroon	Peanut	7	March 1998	4 X E, 1 X D 2 X M
Barkly West	Peanut	2	March 1998	C
Bushbuck Ridge	Peanut	4	March 1998	D
Brits	Peanut	1	March 1998	C
Vaalharts	Peanut	2	March 1998	B
Makathini	Peanut	3	March 1998	F
Makathini	Sunflower	5	May 1998	4 X F, 1 X G
Bergville	Soybean	9	June 1998	I
Bloemfontein	Beetroot	1	August 1999	J
Vaalharts	Peanut	1	January 2000	D
Jacobsdal	Carrot	1	January 1999	E
Jacobsdal	Peanut	3	February 2000	B
Viljoenskroon	Peanut	2	January 2000	1 X F, 1 X E
Ottosdal	Peanut	11	March 1999	7 X C, 2 X B 1 X H, 1 x F
Brits	Peanut	4	March 1999	2 X H, 1 X E1 X L
Hoopstad	Peanut	6	November 1998	4 X E, 1 x B1 X D
Krugersdorp	Valerian	2	December 1998	1 X C, 1 X J
Potchefstroom	Sunflower	1	March 2000	K
Vaalharts	Peanut	9	April 2000	8 X C, 1 X B
Vaalharts	Peanut	1	February 2000	B
Viljoenskroon	Peanut	5	May 2000	2 X C, 1 X D1 X G, 1 X H
George	Carrot	1	Unknown	H

Figure 1: Mycelial interactions between three isolates of *Sclerotium rolfsii*. A compatible reaction occurs between 'top left' and 'top right' (same mycelial compatibility group) and an incompatible reaction occurs between 'top right' and 'bottom' and 'top left' and 'bottom' (different mycelial compatibility groups)

A substantial degree of genetic variation exists within South African isolates of *S. rolfsii,* as reflected by the MCGs identified from different hosts at different localities. This is consistent with the genetic variation identified in an earlier study (Cilliers *et al.* 2000). It has since been reported that a number of MCGs can occur within a specific geographic area or on the same host. Conversely, distribution may be limited to a specific group for a specific area (Punja and Grogan 1983a, Stenlid 1985, Brayford 1990, Leslie 1993, Punja and Sun 1997). South African MCGs do not appear to be strictly linked to a specific host or geographical area. A number of isolates, however, grouped according to host or locality.

Isolation of the same MCG from diverse geographical areas or hosts could be attributed to spread by agricultural practices (Harlton *et al.* 1995), in particular via soil or seed. Results from this study could support this suggestion since there was generally a greater variety of MCGs collected from areas with high agricultural activity, such as Potchefstroom and Viljoenskroon. MCGs represented by single isolates could have resulted from either geographic isolation (as is possible in the case of MCG I and MCG A) or recent colonisation, as suggested by Harlton *et al.* (1995).

It may also be true that an isolate could fall into a specific MCG according to host plant or survival strategy (either parasitic or saprophytic). An isolate growing saprophytically may, by virtue of the genes expressed in its survival strategy, fall into a specific MCG while the same isolate growing parasitically may group into another MCG. This could explain why Harlton *et al.* (1995) found diverse isolates from their worldwide collection grouping in the same MCG and why there were no clear-cut groupings according to host plant or geographical area in either their or the present study.

Genetic diversity could have occurred through the phenomenon of parasexuality which has been reported in fungi such as *Fusarium* and *Aspergillus* (Kendrick 1985). The mechanism occurs in non-sexually differentiated organs and follows the sequence of plasmogamy, karyogamy and meiosis (Ulloa and Hanlin 2000). According to Kendrick (1985) this phenomenon is probably common among conidial fungi, but the frequency of occurrence is rare (occurring in fewer than one conidium in a million). At present the occurrence and role, if any, of parasexuality in *S. rolfsii* is unknown.

Using MCGs as criterion, there clearly is a degree of genetic diversity within the South African population of this

fungus. There appear to be vague associations between MCG and geographical distribution and host, as has been found by other researchers (Harlton *et al.* 1995, Punja and Sun 1997). All isolates from South Africa showed some degree of genetic dissimilarity, whether they were from the same MCG or not (Cilliers *et al.* 2000). Since there appeared to be vague associations between host plant, geographical area and MCG, this implies that the genetic structure is another factor that could be linked to host plant or geographical area.

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