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Mycelial Compatibility Groups Among the Isolates of *Sclerotium rolfsii* Associated with the Southern Blight Disease of Tomato

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ABSTRACT: *Sclerotium rolfsii* is a polyphagus soil inhabitant and ubiquitous facultative saprophyte that causes southern blight/Collar rot on a variety of plants in tropical and subtropical areas around the world. This pathogen is known to cause disease in more than 500 crops. Southern blight disease is emerging as a significant impediment to tomato productivity. Studies of mycelial compatability groups within the population in a geographical region are important because these also document the changes occurring in the population. Hence different pathogenic isolates of *S. rolfsii* were obtained from different regions of Tamil Nadu. In the current study, eight isolates were obtained from diseased tomato crops in various areas of Tamil Nadu, and mycelial interaction was studied among them to determine the pathogen's field population. Only 21 of the 46 pairings produced a compatible reaction. The eight isolates had 48.45 per cent vegetative compatibility based on mycelial compatibility grouping.

Keywords: Mycelial compatibility groups (MCGs), tomato, Southern blight, vegetative compatibility.

INTRODUCTION

S. rolfsii is also one of the most destructive fungal pathogens in terms of host range, capable of causing disease on 500 plant species across 100 plant families, including several economically important crops such as tomato, potato, pepper, cantaloupe, celery, carrot, cabbage, bean, eggplant, and peanut, as reported by (Mullen, 2001). Disease caused by *S. rolfsii* is known as Collar rot, southern blight, Sclerotium root rot, or southern stem rot depending on the plant (Aycock, 1966). In the southern United States, the disease has long been a major issue in the production of row crops such as peanuts. However, the disease has become more problematic in vegetable production as a result of the use of alternative fumigants to methyl bromide, as well as the adoption of organic and other low-input production strategies (Rosskop *et al.*, 2005). The losses caused by this disease can be significant. For example, yield losses in tomato production due to *S. rolfsii* typically do not exceed 25% and are around 7 to 10% on average per year, but losses can reach up to 80% in severe disease years in the southern United States. To effectively manage areas infested with southern blight or collar rot, knowledge of pathogen diversity would provide valuable information on population structure, dispersal, and distribution. Mycelial compatibility refers to the ability of two strains of the same fungal species to fuse and form a single colony, whereas incompatible strains are unable to do so. Mycelial compatibility is one of several events associated with vegetative incompatibility and has been widely used to study potential genetic diversity within field populations of various fungi (Glass *et al.*, 2003). Mycelial compatibility group (MCG) assignment, which is based on hyphal interaction between different isolates, is a popular method for describing *S. rolfsii* diversity. In comparison to isolates from different

groups, isolates from the same MCG are assumed to have a similar genetic background (Leslie, 1993). Based on mycelial interactions between isolates, *S. rolfsii* isolates can be classified into different mycelial compatibility groups (MCGs). MCGs play an important role in defining field populations of fungi and facilitating genetic variation in fungal species where the sexual reproductive stage (teleomorph stage) has little impact on the disease cycle (Kohn *et al.*, 1991). Mycelial (vegetative) compatibility/incompatibility is a self-/non-self recognition system controlled by multiple loci, but understanding of the underlying genetic mechanisms in most filamentous fungi is limited (Glass and Kaneko, 2003). Cultivation of resistant varieties is the ideal and feasible disease control method, and no resistant varieties against this disease have yet been identified. Erect cultivars are more resistant to disease, and crop management can help to reduce crop losses. Due to the prevalence of virulent *S. rolfsi* isolates, stable resistance could not be achieved (Sarma *et al.*, 2002). Previous research has shown that there is variation among *S.rolfsii* populations all over the world (Harlton *et al.*, 1995; Nalim *et al.*, 1995; Okabe *et al.*, 1998). Studies of variability within a population in a geographical region are important because they document population changes. The current study sought to better understand the variability in cultural morphology, sclerotium formation, and mycelial compatibility of *S. rolfsii* isolates collected from various infected tomato plants in Tamil Nadu.

The goal of this study was to identify the collection and isolation of *S. rolfsii* isolates in different districts of Tamil Nadu, as well as to detect their virulence and mycelial compatibility groups using fungal isolates *in vitro*.

MATERIAL AND METHODS

Collection and isolation of *Sclerotium* isolates. Collar rot-infected tomato plants were collected from various fields in Tamil Nadu, including Trichy, Nammakal, Salem, Madurai, Tindivanam, Villupuram, Hosur, and Krishnagiri. The collar rot-infected tomato plants collected during the disease survey were washed with tap water at first. The collar or stem region displaying typical disease symptoms was cut into small pieces. For 30 seconds, these pieces were surface sterilised with 0.1 percent mercuric chloride solution. These pieces were thoroughly washed in sterile distilled water three times to remove any traces of mercuric chloride before being aseptically transferred to sterilised potato dextrose agar (PDA) plates. They were incubated at 27±1°C for three days to allow the fungus to grow. Later, fungal growth loops were transferred to PDA slants. Under aseptic conditions, the fungus was further purified using the hypal tip method (Rangaswamy, 1972).

Determination of MCGs: To determine the MCGs of eight isolates obtained from various tomato growing regions in Tamil Nadu. A 5mm disc was cut from a five day old actively growing culture from each isolate, placed in petridishes as pairings, and cultured on PDA medium at 25±2^{o}C. The presence or absence of a barrage zone where hyphae collide determined the compatibility of each isolate. If the barrage zone was present, it was regarded as incompatible, unless otherwise specified (Punja and Grogan, 1983). The two isolates were kept 40mm apart and incubated on PDA medium at 25±2^{o}C for 10-14 days. (Singh and Singh, 2021).

RESULTS AND DISCUSSION

A disease survey was carried out in major tomato growing areas of Tamil Nadu, India, during the kharif season (June-October 2020). Tomato (*Solanum lycopersicum* L.) plants exhibiting distinctive symptoms of collar rot disease were collected and isolated from various tomato growing areas in Tamil Nadu, including Trichy, Nammakal, Salem, Madurai, Tindivanam, Villupuram, Hosur, and Krishnagiri. A list of the isolates used in the study was provided in (Table 1, Plate 1). There were 36 parings (Table 2) of eight isolates, with only 23 showing compatible reactions and the remaining 13 showing incompatible reactions. The compatible reactions revealed that two paired isolates were intermingled at the interaction zone. Incompatible reactions result in the formation of a thin band of dead mycelium between the two pairings. Based on this observation, 46.45 percent of the isolate pairings were vegetatively compatible. Sclerotia were not formed in any of the incompatible reactions. Sclerotia formed at the lytic zone boundary of two isolates. After prolonged incubation, the interaction zone was observed to broaden in a few combinations. The Sr-A1 (Trichy) isolate is only compatible with the Sr-C3 (Salem) isolate, whereas the Sr-E5 (Tindivanam) isolate has incompatible reactions with all other MCGs. Five distinct isolates of *S.rolfsii* were obtained from various crops. From infected fields, specimens with typical symptoms of southern blight were collected (Ahmed *et al.*, 2019). (Saraswathi and Madhuri, 2013) discovered *S.rolfsii* in groundnut fields around Andhra Pradesh, India, during a survey of groundnut fields. *S.rolfsii* was found to be linked with injured hypocotyls regions of groundnut and plant showing southern blight symptoms were brought to the laboratory using tissue segmentation method on PDA medium. During the survey, wet root rot of sunflower caused by *S. rolfsii* was observed at the grain filling stage in all regions. Dindugal district had the highest incidence of stem rot (5.5 percent), followed by Erode district (5 percent). The Tuticorin district had the lowest incidence (Pandi *et al.*, 2017). The overall findings of this study revealed that *S. rolfsii* populations on tomato in Tamil Nadu were a heterogeneous mix of MCGs. This is consistent with previous reports on the population structures of *S. rolfsii* MCGs on various crops (Li *et al.*, 2008). This study also found that different districts of *S. rolfsii* from

tomato fields contained a variety of MCGs. Similarly, in Canadian studies of *S. rolfsii* on canola (Hambleton *et al.*, 2002) and on soybean in Argentina, this heterogeneous mix of MCGs was observed. (Harlton *et al.*, 1995) screened a global collection of *S. rolfsii* isolates and found 49 MCGs out of 119. (Punja and Grogan, 1983) demonstrated that *S. rolfsii* could be classified into MCGs based on mycelial interactions, identifying 25 groups from 72 isolates. When field isolates of *Sclerotium rolfsii* were paired against each other in culture and used to designate mycelia interaction (compatibility) groups, an aversion reaction developed. The total number of MCG for a global collection of 132 isolates was divided into 71 groups. Additional isolates will undoubtedly increase the number of MCG in this species in the future. There was no clear relationship between host of origin and MCG, which reflects the pathogen's extremely broad host range (Punja, 1988). According to (Punja and li-juan, 2001) the development of aversion zones between *Sclerotium rolfsii* isolates on PDA was visible within 14 days. Initial hyphae intermingling between incompatible isolates was followed by lysis, and a clearing zone quickly formed in the region of interaction. Twelve MCGs were identified among the 459 isolates of *S. rolfsii* (Remesal *et al.*, 2012). Mycelia compatibility among MCG isolates was defined by their mycelia intermingling at the zone of interaction in the absence of a version. In contrast, isolates assigned to different MCGs demonstrated aversion at the interaction zone, as well as thinned mycelium and the formation of a red reaction line. All 459 isolates tested positive for self-compatibility, exhibiting a reaction similar to compatible isolates. MCGs a version zone was observed between incompatible isolates after 7 to 10 days of growth, according to (Xie *et al.*, 2014). When the isolates first came into contact, the hyphae lysed and died, leaving a clearing zone. Over the same time period, colony boundaries of compatible isolates were difficult to discern. (Unal *et al.*, 2019) also stated that all isolates were compatible with one another. At the junction, sclerotia formed. Mating type analysis of 32 isolates revealed that they were all compatible with one another. Mating type analysis of 32 isolates revealed that they were all compatible with one another. As a result, only one mycelial compatibility group (MCG) was observed in *S. rolfsii* in turfgrass.

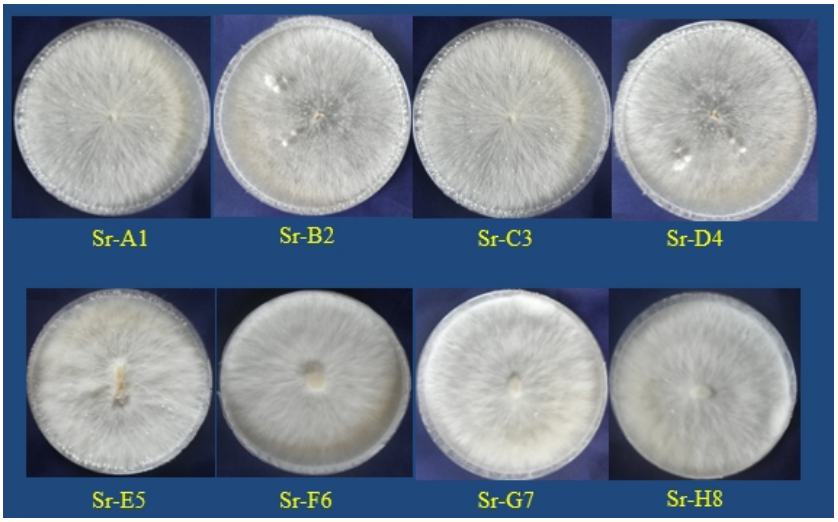

Plate 1: Different isolates of *S.rolfsii* collected from various districts of Tamil Nadu.

Table 1: Isolates of *S. rolfsii* collected from different tomato growing areas of Tamil Nadu.

Sr. No.	Isolates	Host tissue	Location
1.	Sr-A1	Collar region	Trichy
2.	Sr-B2	Collar region	Nammakal
3.	Sr-C3	Collar region	Salem
4.	Sr-D4	Collar region	Madurai
5.	Sr-E5	Collar region	Tindivanam
6	Sr-F6	Collar region	Villupuram
7	Sr-G7	Collar region	Hosur
8	Sr-H8	Collar region	Krishnagiri

Table 2: Mycelial compatibility interaction reaction shown by eight isolates associated with southern blight of tomato.

Sr. No.	Sr-A1	Sr-B2	Sr-C3	Sr-D4	Sr-E5	Sr-F6	Sr-G7	Sr-H8
Sr-A1	C	NC	C	C	NC	C	NC	C
Sr-B2		C	NC	C	NC	C	C	C
Sr-C3			NC	NC	NC	C	C	C
Sr-D4				NC	C	C	C	C
Sr-E5					C	C	C	C
Sr-F6						NC	NC	C
Sr-G7							C	NC
Sr-H8								NC

C- Compatable; NC- Non-Compatable

CONCLUSION

Finally, our findings on pathogen aggressiveness revealed no relationship between morphological characteristics and mycelial compatibility grouping. Morphological characteristics were linked to mycelial compatibility grouping. More research comparing genetics and virulence across populations of different host species growing in close geographical proximity would be useful. This includes testing for resistance to this broad-spectrum pathogen, which is a major disease causer and yield reducer in tomato fields all over the world, including Tamil Nadu.

FUTURE SCOPE

Understanding the population structure and variation in the virulence of *S. rolfsii* will be useful in developing disease management strategies.

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Conflicts of interest. None.

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