

Original Research Article

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Temperature Adaptability and Disease Development Potentiality of Multi-Generation Isolates of *Sclerotium rolfii*

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ABSTRACT

Sclerotium rolfii, obtained from cucumber roots, grew up to 20 generations at 25°C and at 35°C to assess the effect of enhanced temperature on the growth pattern of the fungus. At 25°C, the isolates of early generations covered the entire plate (90 mm) on 120 h after inoculation. However, after the 8th generation, due to repeated isolation and inoculation, reduced mycelium growth observed and almost ceased after the 16th generation. At 35°C, the mycelium started to be visible after 96 h of inoculation but the growth found to be meagre. At this incubation temperature, mycelium attended the progress after 5th generation; a similar response noticed for the 5th to 15th-generation isolates. The adaptability in the growth of this fungus towards higher temperature shows its potential to survive in the changing environment as a result of climate change. Further, the comparison of pathogenicity between the 1st and 20th generation isolates was tested on moong (*Vigna radiata*). Across varieties, no effect of 1st generation-and 20th generation-isolate on collar rot disease development was noticed. But yielding a similar amount of disease by the 20th generation-isolate, which is resistant to elevated temperature, is indicating to take caution that should be implemented in future to make strategies against *S. rolfii*.

Keywords

Collar rot, Disease, elevated temperature, *Sclerotium rolfii*, Temperature adaptability

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Introduction

Sclerotium rolfii causing collar rot is a well-known, non-target pathogen having wide host range. It is among one of the most disparaging

soil inhabiting pathogens and causing significant loss in several crops. The fungus is predominantly distributed in subtropical and tropical countries. It is common where temperature arises during the rainy season.

Environmental condition of an area influences the growth of pathogen and hence the resulting losses (Elad and Pertot, 2014). Previous literature suggests that the optimal germination, growth and sclerotia production of the fungus is best suited at 25°C (Lin *et al.*, 2009). Increase in temperature leads to change in shape and size of sclerotia but not complete discontinuation in the formation of this resting structure. In California, this pathogen may remain active at relatively higher temperatures of 27°C - 32°C (Browne *et al.*, 2002). In Tunisia, the ideal growth can be seen at 30°C-35°C (Daami-Remadjet *et al.*, 2010). The fungus *S. rolfsii* exhibits invariable change in mycelial growth at 25°C. Hence, there is a possibility for adaptation of this pathogen to increased temperature regime (35°C). This could be correlated with possibilities to increase in aggressiveness of this pathogen in the coming decades with higher mean global temperature. The influence of increased temperature due to climate change, on plant disease is hazardous to be through both the host plant and the pathogen (Coakley *et al.*, 1999; Garrett *et al.*, 2006). Climate change in terms of enhancing global temperature will change the pattern of disease by changes in host distribution and phenology, changes in microflora of host and direct biological effects on rapidly adjusting pathogens. An increase in the average annual temperature by 3°C is expected over the next coming century. Atmospheric CO₂, a major greenhouse gas, has increased by nearly 30% and temperature has risen by 0.3 to 0.6°C (Chakraborty, 1998). Plant disease dynamic resulting due to this long term environmental conditions are less studied as the emphasis is considered towards the urgency of problems related to plant diseases. Assessment of the limited literature in this area proposes that the most likely impression of climate change will be felt in three areas: in losses from plant diseases, in the efficacy of disease management strategies and in the

geographical distribution of plant diseases. There may be positive, negative or no impact on individual plant diseases due to change in temperature. The increase in temperature, as an outcome of climate change alters the behavior of both host plant and the pathogen. Adaptability of *S. rolfsii* changing environmental conditions such as high temperature regimes are important to study.

Materials and Methods

This is an extensive work of our previous attempt (Kumar *et al.*, 2017). The fungus was sampled from infected cucumber root, Agricultural Farm of Bihar Agricultural University, Sabour. The infected plant was taken to the laboratory, washed properly under flow of water, cut into small piece of 5mm which include both infected and healthy portion. The segment was then surface sterilized by 1% sodium hypochloride for 2 min and rinsed in sterile distilled water thrice. The segment was allowed to grow on PDA (potato, dextrose, agar: 200 g, 20 g, 20 g in 1-liter water) media supplemented with streptomycin antibiotic to check any type of bacterial growth at 25±1° C for 7 days. The fungal mycelia were taken aseptically by inoculation loop and transferred to PDA slant for pure culture of the fungus. The pure isolate was identified on the basis of various characteristics (Sarma *et al.*, 2002). The pathogen was subjected to grow under two different incubation temperatures i.e. 25°C and 35°C. A disc of actively growing mycelium was placed at the center of petriplate and cultivated for 120 h (at 25°C) when the mycelium touched the periphery of the plate (Muthukumar and Venkatesh, 2013). The mycelium was re-isolated up to 20 generations. Four replicates for each temperature regime in a generation were adjusted following a complete randomized design (Gomez and Gomez, 1984) and the whole experiment was repeated twice. The

comparison of fungal growth rate was made between 25°C and 35°C for the acclimatization of the pathogen in successive generations in response to climate change in the form of increased temperature.

An experiment on artificial inoculation was conducted to study the disease development potentiality of the pathogen using isolates of two generations (1st and 20th). For that, the assessment of collar rot disease in moong (*Vigna radiata*) was done after growing in root trainer under open condition during middle February to April of 2019. The garden soil was previously subjected to double autoclaved in polypropylene bag containing 1.5 kg soil in order to eliminate the effect of any other soil-borne microorganism. Root trainers were filled-up with the sterilized soil mixed with the sclerotia of *Sclerotium rolfsii* isolates at the rate of 100 sclerotia per 100 g of soil (Yaqub and Shahjad, 2005). Sowing was done using 4 different popular varieties viz. Samrat, Pusa Vishal, Hum-16 and Meha. The experiment was repeated twice. The experiment was conducted in Complete Randomized Design (Gomez and Gomez, 1984) involving 5 replications. Two root trainers (total 24 plants) were considered for each replication.

Results and Discussion

Effect of two different temperatures on the growth pattern of *Sclerotium rolfsii* and its pathogenicity in the successive twenty generations obtained by sub-culturing was assessed.

Alteration in the growth pattern of *Sclerotium rolfsii* during successive generation

The percent change in mycelial growth of the pathogen was found to be in increasing order (increase in mycelial growth than the previous

generation) at a higher temperature (35°C; figure 1). A reverse trend was observed at 25°C. A decrease in mycelial growth than the previous generation was noticed at 25°C. Up to 5 generations, no change in the growth pattern of mycelia was observed as compared to the previous generation at 25°C. After 5th generation, retardation in the percent change of mycelial growth was seen after 120 h. This change was more prominently seen in a negative direction after 10th generation and a similar difference was observed after 15th generation. At a higher temperature of 35°C, a slight increment in the percent change in mycelial growth was observed up to 15th generation, but a large positive raise was observed after 15th generation (figure 1). The change was found to be significant ($p \leq 0.001$) after 16th generation at 35°C.

At 25°C, the radial growth in the first generation started after 24 h and the full plate growth was seen after 120 h. After frequent sub-culturing i.e. growth of the pathogen generation after generation at the same temperature, it was observed that in the 20th generation reduced radial growth was noticed after 24 h as compared to the radial growth after 24 h in the first generation (figure 2). At 35°C, the radial growth in first-generation was seen only after 72 h. Nearly 20 mm radial growth was observed after 120 h. At higher temperature i.e. 35°C, in the 20th generation, the radial growth at 96 h was found to be more than as seen in the first generation and the same trend was seen after 20th generation at 120 h. It is just reverse to that of 25°C (figure 2). At optimum of 25°C, after successive sub-culturing, generation after generation the growth of pathogen was found to be in a retarding manner but at the higher temperature, the growth was found to be in an increasing mode. This shows the adaptability for the survival of the pathogen at higher temperature and changes at their optimum temperature. Plant pathogens have three

general possibilities related to adapt to a changing environment: (i) exploit the present phenotypic plasticity, (ii) migrate to areas with a suitable climate, and/or (iii) evolve new characteristics (Oliva *et al.*, 1993). Plant pathogens can grow and reproduce across a wide range of temperatures and can tolerate large thermal variations in different environments. Correlation can be made between the increased global temperature and impact on the disease caused by this fungus. Climate change presents considerable challenges for plant disease management (Garrett *et al.*, 2006). While the particular effects of climate change on plant diseases will depend on the plant-pathogen system. The impact changes, such as temperature can be predicted for several plant species from empirical studies (Coakley *et al.*, 1999).

Invariable mycelial growth at different temperatures

To estimate the impact of temperature on growth of *S. rolfsii*, the fungal isolate was grown on PDA and radial growth of mycelium was observed on 24, 48, 72, 96 and 120 h after inoculation (hai) at two different regimes of temperature i.e. 25°C and 35°C. In general, the pathogen grew at 25°C and touched the periphery of the petriplate (90 mm) on 120 h. At 25°C, no mycelial growth was recognized up to 24 h; the visible growth of the fungus was observed on 48 h and completely filled the petriplate on 120 h. The fungal growth measured on 72 h incubation was double of the growth taken on 48 h incubation. While the 96 h incubated petriplates rendered 1.5 times increase of mycelial growth over 72 h incubated petriplates. Similarly, the 120 h incubated petriplates had nearly 1.2 times progress of mycelium than 96 h (figure 2), which is in support with the earlier work done by Kumar *et al.*, 2017. A reduction in fungal growth was observed starting from the 8th generation at

25°C. A significant difference in the growth reduction was recorded for incubation period 48 h, 72 h and 96 h. However, at the 16th generation, a meagre reduction in the growth of mycelium was detected; on 120 h the mycelial growth was restricted to 87 mm, which was 90 mm in the isolate of 1st generation (figure 2). This is indicating that the growth of the fungus is invariable to this temperature regime.

Lower fungal growth was recorded for high incubation temperature in initial generations. At 35°C, no mycelial progress was observed even after 72 h of incubation at initial generations (figure 2). Even after 96 h incubation, poor growth (< 10 mm) was noted and app. 20 mm growth was observed after 120 h. Up to 4th generation, the fungal growth was just double when the comparison was made with the two incubation period of 96 h and 120 h. The 5th and 6th generation envisaged with the progress of approximately 2.5 times of fungal growth at 120 h than the growth at 96 h (figure 2). In the isolates of successive generation, the mycelial growth was recorded higher at the incubation period of 96 h and simultaneously at 120 h. At higher temperature, an increase in the trend of mycelial growth was detected onward to 5th generation (figure 1). This could interpret with the adaptability of *S. rolfsi* to increased temperature regime (35°C). Temperature and generations both had significant ($p \leq 0.001$) role in the changing of normal growth trend of the pathogen and adaptability to the new environment i.e. higher temperature regime. Adaptation to higher temperatures results mainly from genetic differentiation between populations (Zhang *et al.*, 2011), but the presence of widespread plasticity in response to thermal extremes in these fungal pathogens suggest that the lack of genetic variation will not necessarily limit species distribution under climate warming. Adaptive plasticity may also contribute directly to species

intrusiveness by allowing rapid colonization of diverse new hosts without the need to undergo the local selection (Williams *et al.*, 1995). Individual plasticity may have an impact on the patterns of evolutionary diversification at the population (and ultimately species) level by excluding selective deviation in environmentally distinct sites (Sultan and Spencer, 2002).

Pathogenicity of 1st and 20th generation isolates of *Sclerotium rolfisii*

The pathogenicity of *S. rolfisii* isolates of 1st and 20th generation were assessed using four popular moong varieties viz. Samrat, Pusa Vishal, Hum-16 and Meha. *S. rolfisii* epidemics initiated in the root trainers by artificial inoculation (Yaqub and Shahjad, 2005). Disease incidence (DI) was recorded in moong after inoculation of *S. rolfisii* isolates of 1st and 20th generations as suggested by Ghatak *et al.*, 2010. An isolate of 1st generation of the pathogen showed highest DI observed in Meha followed by Samrat, Pusa Vishal and Hum-16. From table 1, it can be noticed that DI caused by the isolate of 20th generation in the first experiment also rendered a similar trend and the difference in

DI caused by the isolate of 1st generation and 20th generation was found to be non-significant ($p \geq 0.001$). After several sub-culturing, even the virulent isolates loss their degree of pathogenicity (Ansari and Butt, 2011; Eslami *et al.*, 2015; Sultana *et al.*, 2018); the property is known as attenuation.

The presence of virulence in the isolate assists in survival and the non-virulent ones are reduced or eliminated with each re-inoculation (Agrios, 2005). In this study, the isolate showed nearly no difference in its pathogenicity (disease development) whether it has been used after 19th sub-culturing. This shows the high degree of adaptability and virulent nature of the isolate. The adaptive behaviour towards high temperature as seen in the form of changes in radial growth from generation 1 to 20 at two different temperature regimes has an impact on the diseases caused by this fungus, as indicated in producing DI (table 1). Several aspects of the biology of a pathogen can be directly influenced by changing environmental factors (Ghatak and Ansar, 2017; Vati and Ghatak, 2015).

Table.1 Comparison between 1st and 20th generation isolates of *Sclerotium rolfisii* for disease incidence in moong varieties

Moong varieties	Experiment 1		Experiment 2	
	Generation 1	Generation 20	Generation 1	Generation 20
Samrat	22.0 (± 2.8)	23.2 (± 1.1)	21.9 (± 2.3)	21.1 (± 0.9)
Pusa Vishal	21.1 (± 1.3)	19.6 (± 2.0)	20.5 (± 2.0)	19.9 (± 1.0)
Hum-16	10.8 (± 1.3)	11.2 (± 1.1)	11.5 (± 0.9)	12.3 (± 2.7)
Meha	29.4 (± 3.7)	28.9 (± 1.7)	28.9 (± 1.8)	29.0 (± 1.0)

The values inside the parentheses are the standard error of the mean.

Figure.1 Percent change in radial growth (mm) of *Sclerotium rolfsii* successive twenty generations. Bars of particular temperature with similar letter are not significantly different according to Duncan's multiple range test at $p \leq 0.001$

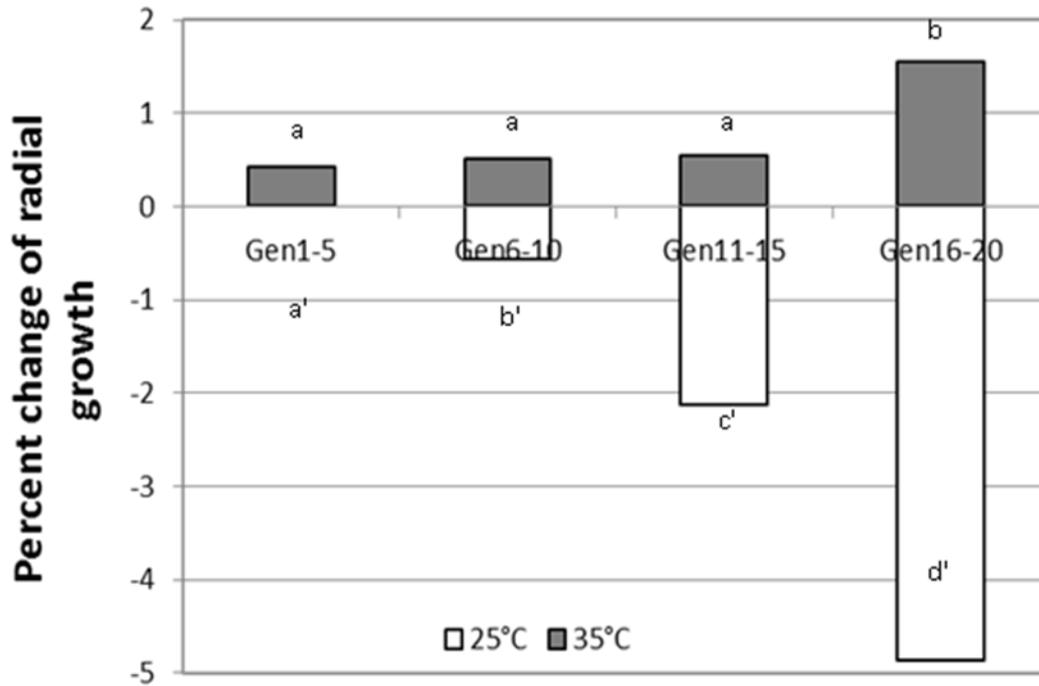
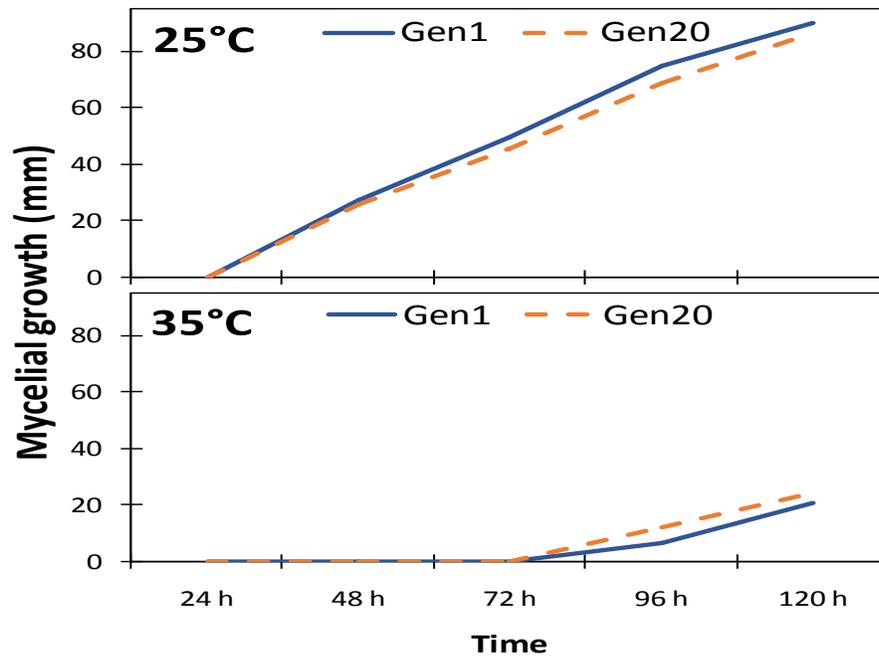


Figure.2: Variation in mycelial growth at different time intervals in two generations. Gen1: generation 1st and Gen20: generation 20th



Continue exposure to an environmental condition like the temperature at its optimum for the development of the pathogen leads to increase in severity of disease-causing ability and hence to high epidemics. With a rise in the duration of ideal temperature, many pathogens spread in a new manner and infect the new hosts in the region. The temperature, in particular, has a primary role in the interaction between pathogen and their hosts, affecting epidemic outbursts and co-evolution processes. Also, some other traits at gene level may be present in the adapting strains of *S. rolf sii*.

In conclusion, at the preliminary stage, the present study unveiled another facet of this fungus. This information opens a scope for further studies considering the temperature adaptability with an increased number of generations. Each pathogen has got its own cardinal temperature and understanding the temperature of the pathogen will help to standardize the management practices. But the adaptability of the pathogens towards higher temperature in the arena of global warming and climate change will disturb various models of crop protection so, from the management side, these needed to be upgraded regularly. The potential influence of global warming on a species will depend on its thermal reaction norm and the underlying genetic dissimilarity for temperature sensitivity in the affected populations (Zhang *et al.*, 2011).

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