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Rhizoctonia solani Kuhn., a devastating necrotrophic pathogen, causes various diseases on tobacco (*Nicotiana tabacum* L.) seedlings as well as on adult plants leading to significant crop loss. The goal of this study was to study differential behavior of the fungus on its host surface versus an artificial growth media. Behaviour of sclerotia of *R. solani* and hyphae emerging from germinated sclerotia were studied on leaf, stem, and root surfaces of SR1 tobacco plants and compared with that observed on artificial growth media. This behavior was analyzed in terms of the time of germination sclerotia, growth of hyphae on surfaces of different plant organs and branching pattern of hyphae on leaves. Sclerotia of *R. solani* showed comparatively slower germination on the surface of SR1 tobacco leaves than media. Growth of hyphae emerging from germinating sclerotia was found to be significantly less on the surfaces of leaves. Among different parts of SR1 tobacco plants highest hyphal growth was found on leaves and lowest on stem at 24 hpi. Branching pattern of *R. solani* hyphae on artificial media was more complex with higher amount second and third degree branches compared to SR1 tobacco plants. The differential behaviour of hyphae of *R. solani* on different parts of SR1 tobacco plants versus on media was likely due to non-availability of ready nutrients compounded with defense structures or chemicals present of the host surface.

Key words: Artificial media, branching pattern, hyphal behaviour, *Rhizoctonia solani*, sclerotia, tobacco SR1,

INTRODUCTION

Tobacco (*Nicotiana tabacum* L.) is a widely cultivated important cash crop worldwide as well as in India (Rizwan *et al*. 2019). Tobacco farming and tobacco related industries are a big contributor to Indian economy.

Rhizoctonia solani Kühn is a devastating necrotrophic fungal pathogen that causes various diseases in different crops (Basu *et al.* 2016, Saha *et al*. 2022).This fungus causes different diseases in tobacco plants at seedling stage viz. damping off and stem rot which causes death of upto 100% seedlings (Gonzalez *et al*. 2011; Sturrock *et al*. 2015; Gveroska, 2019). *R. solani* infection also results in diseases like root rot, target spot, sore shin in mature tobacco plants that can lead to huge yield loss (Gonzalez *et al*. 2011; Zhu *et al.* 2022). Diseases caused by *R. solani* led to upto 50% crop loss worldwide annually (Chen *et al.* 2016).It was shown in earlier studies that hyphal growth of many pathogenic fungi like *Alternaria solani*, *Fusarium oxyspoum*, *Botrytis cinerea*, *Sclerotinia sclerotiorum* as well as on non-pathogenic fungi like *Pleurotus* and *Ganoderma* and different arbuscular mycorrhiza have been affected by nutrient status of the growth media i.e. nutrient content and nutrient sources and favourable growing condition i.e. susceptible host or nutrient rich condition (Temme *et al*. 2012, Weikl *et al*. 2016; Lane *et al.* 2018; Fletcher 2019;Pan *et al*. 2020). Germination of sclerotia and hyphal growth of *R. solani* are also greatly affected by nutrient sources and nutrient condition (Ritchie *et al.* 2009; Saha *et al*. 2022). Previous studies from our laboratory on *R. solani* and *A. solani* have showed different nutrient condition and sources

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and other abiotic factors like nutrients, light intensity and light duration regulated hyphal growth and hyphal branching (Saha *et al.* 2022). Earlier studies from our laboratory also have reported that different fungi like *A. solani*, *Magnaporthe oryzae* have showed differential hyphal growth in the vicinity of susceptible and tolerant host tissue (Ray *et al.* 2015, Basu *et al*. 2016; Mondal *et al.* 2017; Koley *et al.* 2022).

But there are no studies on the comparative behaviour of germinating sclerotia and hyphae of *R. solani* between artificial media and tobacco plant surface. In this present study, we have analyzed the comparative germination of *R. solani* sclerotia on artificial media and tobacco plants. We have also analyzed differential behaviour of *R. solani* hyphae on different parts of tobacco plants and artificial media.

MATERIALS AND METHODS

Plant material

Tobacco (*Nicotiana tabacum* L.) cv. petit havana SR1 was used in this study. Tobacco plants were grown in pots containing soilrite (mixture of peat moss, horticulture grade perlite and exfoliated vermiculite in 1:1:1 ratio)in plant growth chambers maintained at 26°C ambient temperature. Six week old plants were used for further experiments.

Fungal material

Rhizoctonia solani Kühn (isolate AG1-IA) that causes root rot and damping off on tobacco plants was used as the fungal pathogen in present study. This fungus was grown and maintained in potato dextrose agar (PDA) medium at 28°C. Mature sclerotia taken from full grown cultures were used as inocula according to Basu *et al*. (2016).

Study of germination of R. solani sclerotia on artificial media and tobacco leaves

Artificial growing condition i.e. high carbon and low nitrogen with potato and dextrose as source of carbon and nitrogen (potato dextrose agar, PDA) was used. For tobacco plants detached leaf assay was performed. Artificial media and leaves of tobacco plants were inoculated with sclerotia from *R. solani* culture plates by placing a single sclerotium on the middle of tobacco leaves and artificial growth media. Germination of sclerotia on these experimental conditions was analyzed upto 24 h post inoculation (dpi).

Comparative study of growth of R. solani hyphae on artificial media and tobacco plants

For this study, different parts of tobacco plants viz. stem, leaves and roots were inoculated by placing *R. solani* sclerotia on the surface. Growth of hyphae of *R. solani* on these different parts of tobacco plants were analyzed upto 72 hpi by measuring length of hyphae from germinating sclerotia. This growth of *R. solani* hyphae on tobacco plant was compared with that observed on artificial growth media.

Comparative analysis of branching pattern of R. solani hyphae on artificial media and tobacco plants

For this study detached leaf assay was used. Artificial media and leaves of tobacco plants were inoculated with *R. solani* by placing a single sclerotium in the center of culture plate or leaf. Branching pattern of emerging hyphae from germinated sclerotia was observed under compound microscope (Zeiss, Germany) and number of branches of different degrees was documented.

Statistical analysis

For statistical analysis Graphpad Prism software version 7 for windows was used. For the comparative analysis of hyphal growth on artificial growth media and on different parts of SR1 tobacco plants, data from three independent experiments with three replicates were used. Analysis of variance (ANOVA) was done to test significance of the data. Data was presented as mean ± standard error of mean (SEM). $P < 0.05$ was considered as significant difference between different groups.

RESULTS AND DISCUSSION

Differential germination time of R. solani sclerotia on artificial growth media and SR1 tobacco leaf surface

The behaviour of sclerotia of *R. solani* after inoculation on to surface of SR1 tobacco leaves was compared with their behaviour on artificial

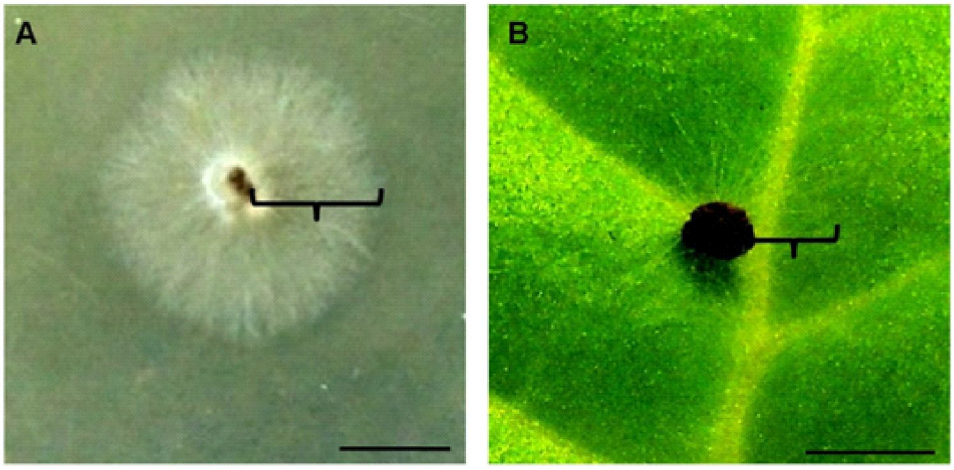

Fig. 1: Germination of sclerotia of *Rhizoctonia solani* on artificial growth media and on SR1 tobacco leaf surface.(A) Germination of sclerotia on artificial media at 12hpi. Bar = 3cm (B) Germination of sclerotia on tobacco leaves at 24hpi. Bar = 1cm

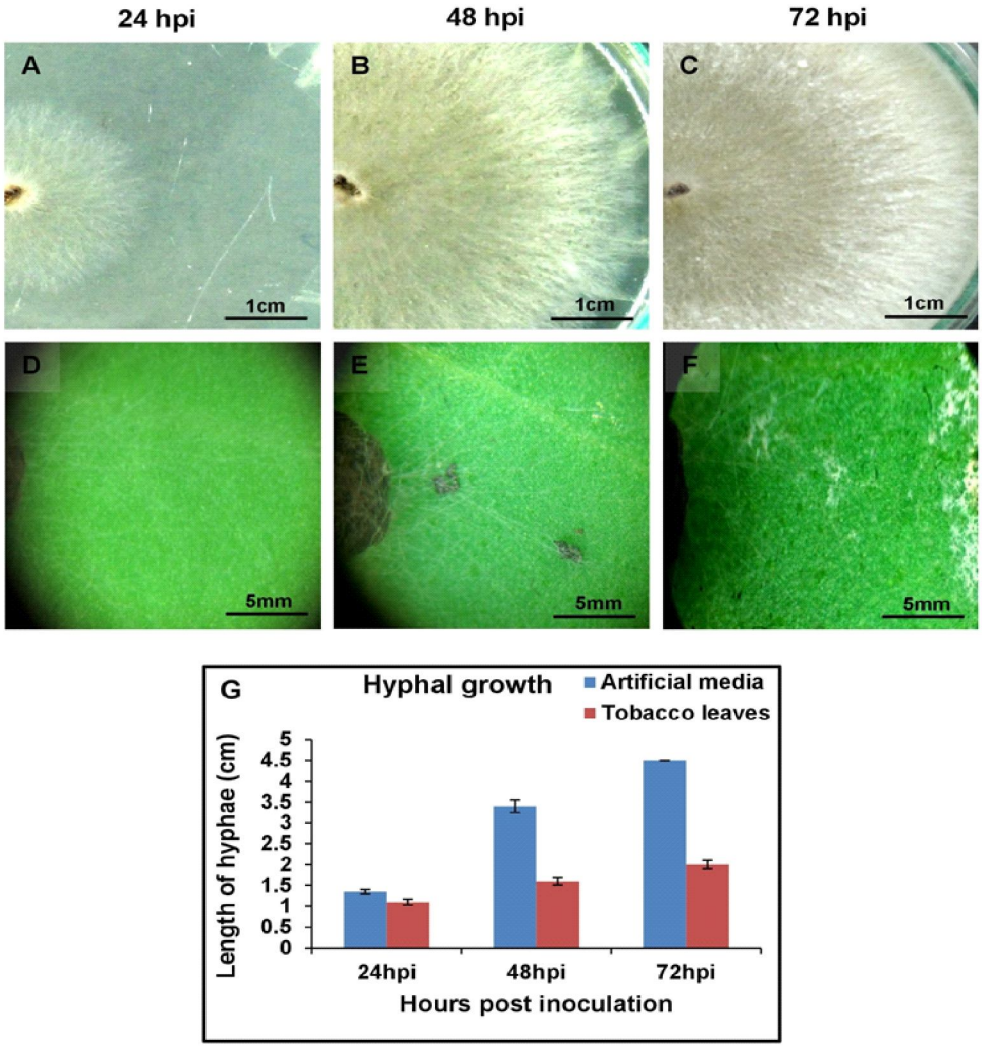

Fig. 2: Comparison of mycelial growth from germinating sclerotia of *R. solani* on artificial growth media and on tobacco leaf surface at 24, 48 and 72 hours post inoculation (hpi). (A-C) Plates showing sclerotial germination and mycelial growth (Length of hyphae) on artificial media. (D-F) Tobacco leaf surface showing sclerotial germination and mycelial growth. (G) Graphical representation of comparison of growth of mycelia (radius of colony) on artificial media and tobacco leaves over 3 day time period post inoculation.

growing conditions. It was observed that germination of sclerotia was earlier under artificial growing conditions compared to that on tobacco leaf surface. The germination of inoculated sclerotia occurred before 12 hpi in artificial growth media and after 12 hpi on tobacco plants (Fig. 1). So, a differential behaviour was observed during germination of sclerotia of *R. solani* between tobacco plants and artificial nutrient media. This is due to non-availability of ready nutrient source and also likely due to the host leaves exudates having antifungal activity. Earlier studies from our laboratory showed that hyphal growth was more retarded on leaves of tolerant host compared to susceptible host (Ray *et al*. 2015; Basu *et al.* 2016; Mondal *et al*. 2017) Ritchie *et al.* (2008) have previously shown differential germination of *R. solani* sclerotia with significantly higher germination on soil than on nutrient less agar media. This also corroborates our results that unavailability of nutrients delays germination and growth of the fungus.

Comparison of behaviour of R. solani infection hyphae emerging from sclerotia on artificial growth media and on surface of SR1 tobacco leaves

We have studied the behaviour of *R. solani* hyphae emerging from germinating sclerotia on artificial medium and surface of leaves of SR1 tobacco plants. Length of hyphae from germinating sclerotia on the surface of tobacco leaves and on artificial media was measured at 24, 48 and 72 hpi. We found that on artificial growth media, length of hyphae from germinating sclerotia were 1.35 cm, 3.4 cm and 4.5 cm after 24, 48 and 72 hpi respectively (Fig. 2A-C). Whereas on leaves of tobacco, length of *R. solani* hyphae at 24, 48 and 72hpi was found to be 1.1 cm, 1.6 cm and 2 cm respectively (Fig. 2D-F). At earlier points after inoculation i.e. at 24 hpi growth of hyphae were almost similar but with increase in time post inoculation i.e. at 48 and 72 hpi *R. solani* showed almost twice hyphal growth in artificial media compared to tobacco leaf surface (Fig. 2G). So, *R. solani* showed differential growth especially at later points of infection. We have previously reported that *R. solani* showed differential hyphal growth under different nutrient conditions and sources (Saha *et al.* 2022). In a previous report *B. cinerea* have been found to show differential hyphal growth on glass slide and on tissues of *Brassica napus* (*Garg et al.* 2010). So, this differential behaviour might be due to varying nutrient source and growing condition. It was also reported from our lab in rice that growth of *R. solani* was higher in the vicinity of susceptible host compared to resistant host (Basu *et al*. 2016).

Differential growth of R. solani hyphae on root and stem surfaces of SR1 tobacco plants compared with artificial growth media

We have also compared the growth of *R. solani* hyphae on stem and root surface of tobacco plants and on artificial media. The growth of hyphae from germinating sclerotia of *R. solani* on roots and stem surfaces after 24 hpi was 0.9cm and 1.15 cm respectively (Fig. 3B, C). So, growth of hyphae was lowest on stems (0.9 cm) and highest on artificial media (1.35 cm) after 24 hpi (Fig**.** 3**)**. In an earlier report we have previously shown differential behaviour of infection cushions and other infection structures in different parts of tomato plants (Koley *et al*. 2022).

Differential branching pattern of R. solani hyphae on artificial growth media and on surface of SR1 tobacco leaves

We have observed the branching pattern of *R. solani* hyphae emerging from sclerotia on the surface of tobacco leaves and compared with that on artificial media. We have found emergence of 14, 7 and 3.5 branches per 100 μm^2 of surface area respectively of first, second and third order of *R. solani* hyphae on the artificial media at 24 hpi (Fig. 4).Whereas, number of first and second order branches observed on tobacco leaves were 5.6 and 2.2 per 100 μm^2 of surface area respectively at 24 hpi (Fig. 4). No third degree branches were observed on tobacco leaves at 24 hpi. So, the number of branches of both first and second degree on the surface of tobacco leaves was found to be significantly lesser compared to artificial media. Therefore, it is apparent that, nutrient rich growth conditions have increased branching of *R. solani* and also supported branching of 2^{nd} and 3^{rd} orders.

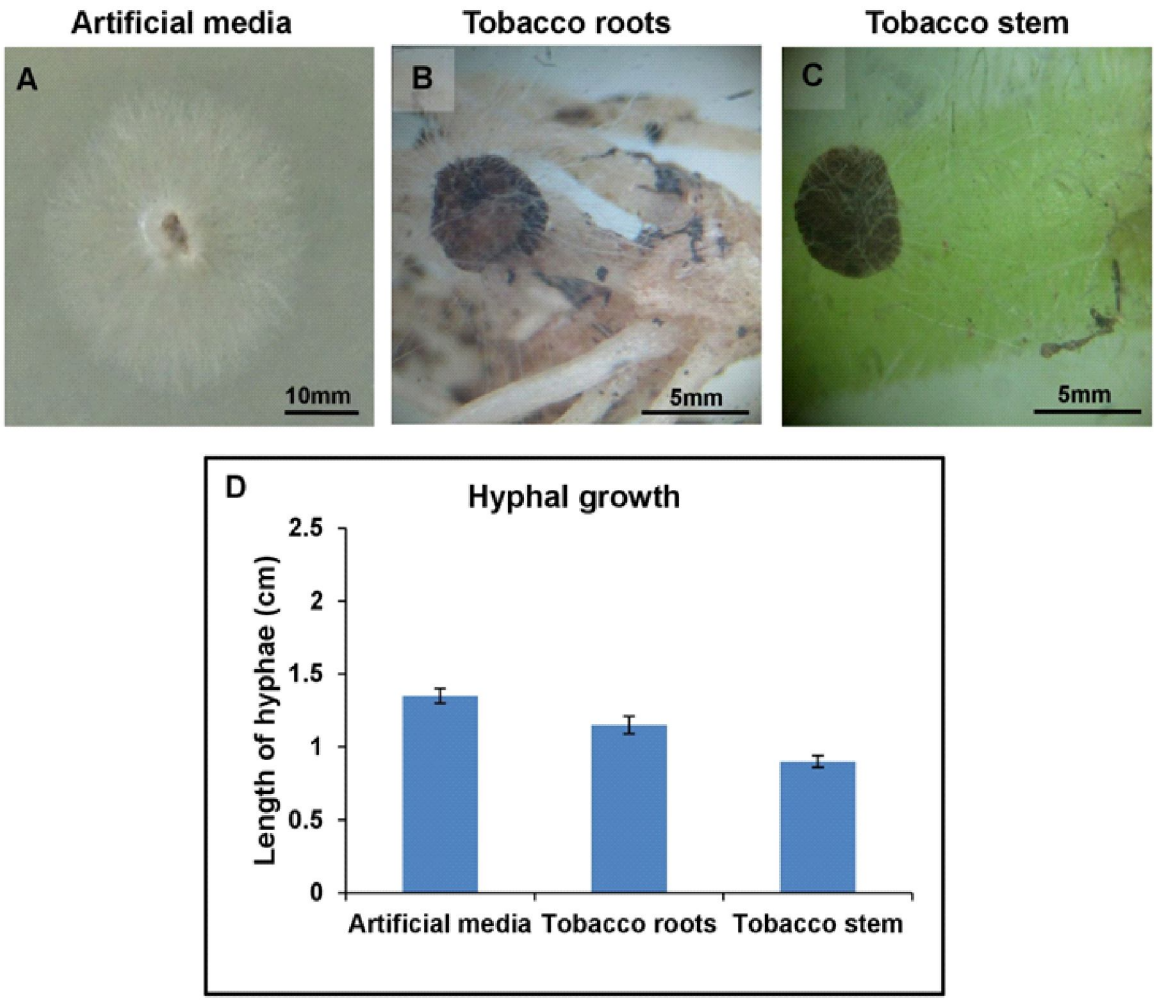

Fig. 3: Comparison of growth of *R. solani* mycelia from germinating sclerotia on artificial growth media and on stem and root surface of tobacco plants at 24 hpi**.** (A) Plate showing sclerotial germination and mycelial growth on artificial media at 24 hpi. (B) Tobacco root surface showing sclerotial germination and mycelial growth. (C) Tobacco stem surface showing sclerotial germination and mycelial growth. (D) Graphical representation of comparison of growth of mycelia (radius of colony) on artificial media and tobacco root and stem surfaces at 24 hpi**.**

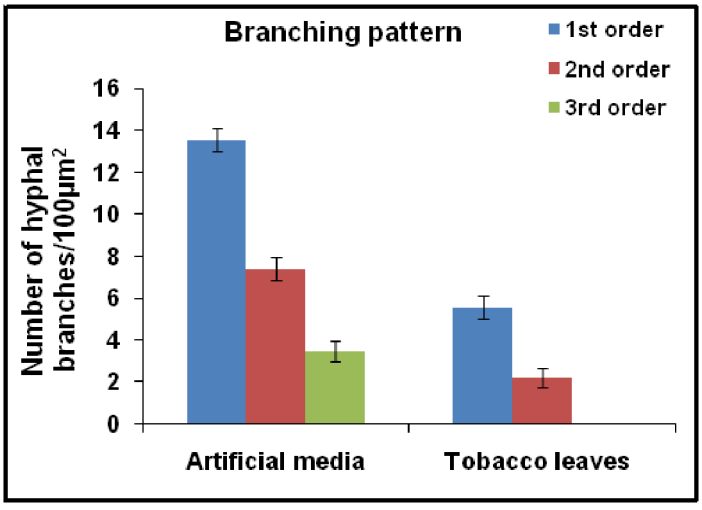

Fig. 4: Comparison of branching pattern of *R. solani* hyphae from germinating sclerotia on artificial media and leaves of SR1 tobacco plants at 24hpi.

CONCLUSION

In conclusion, our study revealed that *R. solani* sclerotia preferred growth media over host surface for germination. Moreover the new hyphae emerging from the sclerotia also proffered growth media than any host surface like leaves, stems and roots and showed faster growth of hyphae. Similarly hyphal branching was less advanced on host surfaces compared to media where almost 3 times more second and third degree branching was visible within 24 hours post inoculation. Although tobacco is a natural host for *R. solani*, the host surfaces lack readily available nutrient sources. The host surfaces also have defense mechanisms that prevent colonization of fungi and thus growth of the hyphae is slower on host surfaces.

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