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Effect of Culture Conditions on Formation and Germination of Sclerotia of *Rhizoctonia solani*

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Abstract
Perception of either continuous light or dark signal regulates the hyphal morphology, growth intensity and branching induction in *Rhizoctonia solani*. Hyphal length is enhanced under nutrient stressed condition. In this present study, the differential response of the AG1-1A isolate of *Rhizoctonia solani* was evaluated on two different media. Growth characteristics, sclerotia production were observed and found to be maximum under continuous exposure to light along with nutrient rich condition.

Keywords: *Rhizoctonia solani, complete dark, complete light, sclerotia, hyphal growth*

1. Introduction

Filamentous form is considered as the most ancestral one besides the unicellular bodies in fungal entity. In case of filamentous fungi, the vegetative body is the result of the cumulative association of the hyphae, unit of vegetative growth. The hyphae elongates at their tip by the inclusion of membrane and cell wall materials, coming from the interior of the cell at the place of fusion through vesicles (Riquelme et al., 2018). Light also play a major role in regulating fungal morphology both in vivo and in culture along with a many of other processes (Babitha et al., 2008). Individual cell length in septate hyphae was known to be increased at elevated CO2 level and with continuous light exposure (Raudaskoski et al., 1982). *Rhizoctonia solani* Kühn. is a potent necrotroph causing threat to a number of crops, some most dangerous examples are sheath blight in rice, black scurf in potato, damping off in tobacco, cotton, cabbage, cauliflower, brinjal, indian spinach and chilli. (Goswami et al., 2011). Being a necrotrophic pathogen, it can grow on dead infected plant parts, produced sclerotia which can survive both in soil as well as water and reach to the next season to start a new cycle (Basu et al., 2016). This pathogen predominates in the vegetative state as mycelium or an asexual stage in the form of sclerotium. Sexual structure is found rarely, which after identification is categorized under basidiomycotina and named as *Thanatephorus cucumeris*. Sexual uninucleate spores are produced on each basidium, only under favorable condition upon getting a high content of moisture. Germinating spores of *Thanatephorus cucumeris* from vegetative hyphae which can fuse with each other to develop mycelium of a variety of nucleus (Taheri et al., 2012). Currently it has been classified into 14 anastomosing group (AG) till date, namely AG 1 to 13 and one Bridging Isolate (BI) (Nikraftar et al., 2012). Not only they vary on the basis of anastomosis, but also over a number of other parameters like growth rate of the colony, colony color, type of zonation, size and number of sclerotia,

saprophytic behaviour and pathogenicity (Dutta et al., 2012, Goswami et al., 2011, Silva et al., 2015). This fungus is well known for production of sclerotium, a structure composed of tight accumulation of melanized hyphae without the cellular differentiation into rind and medulla. This structure can germinate within hours and stared producing vegetative hyphae which can infect directly the nearby hosts. (Ritchie et al., 2009). Many studies have reported on different aspects of sclerotia, it is also reported that sclerotia grow faster on visible light than in the dark, and its production also increased under light (Dutta et al., 2012). Similar facts were also validated much earlier by Singh et al. 2001, in addition they revealed that light from different ranges of visible spectrum were involved in regulating the process of sclerotial formation. Not only physical condition, but also some biotic factors like the association with other fungus indeed alter the sclerotial induction as well as its intensity in many sclerotia producing fungi like *Rhizoctonia solani* and *Bacillus subtilis* (Singh et al., 2001). Continuous illumination elevated asexual spore production over darkness, which is required for sexual structure to develop had found in *A. nidulans* (Bayram et al., 2008). In spite of being an important pathogenic form, there is very few detailed knowledge about the factors affecting sclerotia production. The major aim of this work is to understand how the sclerotia production varies with different environmental conditions.

2. Materials and Methods

2.1 Fungal Material

Rhizoctonia solani Kühn. (AG1-1A isolate, supplied by Rice Research Station, Chinsurah, West Bengal, India) was used in this study as the pathogen. It was maintained as a pure culture. For that, 2% Potato Dextrose Agar (PDA) media were used as medial supplementation. A small inoculum was then transferred aseptically from the main plate to the new solidified PDA plate, and maintained at 28°C at 16-hour light and 8-hour dark photoperiod.

2.2 Preparation of Inoculum

In this work, 10-days-old culture of *R. solani* was used to prepare the inoculum. A 3 mm mycelium disc was scooped from the growing edge of the culture by a sterile cork borer and was used for infection.

2.3 Comparison of hyphal behaviour on PDA and agar media

For this study two different aseptic media were made, one is PDA (Potato Dextrose Agar), a basal nutrient medium and another is 2% agar only, that is nutrient starved media. In order to study in vitro, the behaviour of the pathogen on the above two media, a 3mm mycelium disc was aseptically placed exactly at the centre of the petriplate. Mycelium discs were scooped from the edge of 10-day old culture. Details of hyphal behaviour were observed under compound (Olympus Bx-51) microscope. Growth was observed at three observation points viz- 6 hour, 8 hour and 24 hour post inoculation (hpi).

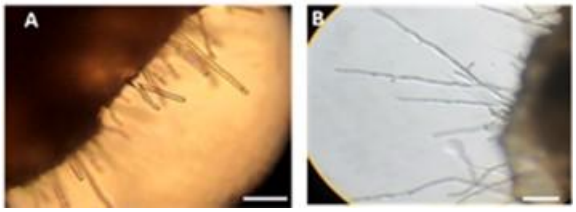
2.4 Differential amount of sclerotia production under PDA and agar media

R. solani inoculum was placed carefully on two petriplate (9 cm) filled with either of the two media PDA and agar. One plate was incubated under complete dark condition and other under complete light. All these plates were incubated in suitable environmental conditions already stated above till they produce sclerotia. The development and number of sclerotia was observed under stereomicroscope and counted at 12 dpi. Three petri plates represent three replicates of one experimental set. Three experiments were done for each media.

3. Results

3.1 Attributes of Growth of *Rhizoctonia solani* under two different nutrient conditions

The phytopathogen is found to take a preparatory lag phase of about 4 hours to start a fresh growth on a new media. After 4-hours new hyphae start to emerging and branch at right angle to the main hyphae. In case of PDA, the hyphae grown from every side of the inoculum disc. They attain a moderate length, branch at short interval and grow more profusely from the very beginning (Figure 1A). On the contrary, at 4-hour, the hyphae on nutrient starved media, (agar) grow more rapidly and straight without making much lateral branch (Figure 1B). The hyphal length on agar was as much two times longer as that of on PDA (Figure 1C). In the subsequent time points also, the trend was followed. Hyphal mat also shows another feature which is the distance between two primary hyphae. Hyphae grown with an average 137.5µm ±11.5 µm inter-hyphal distance in case of PDA where as in case of agar this distance was 285µm ±15.3 µm (Figure 2C). Hence it is evident that mycelial growth was much dense and profuse on PDA basal nutrient medium than agar, nutrient starved medium.



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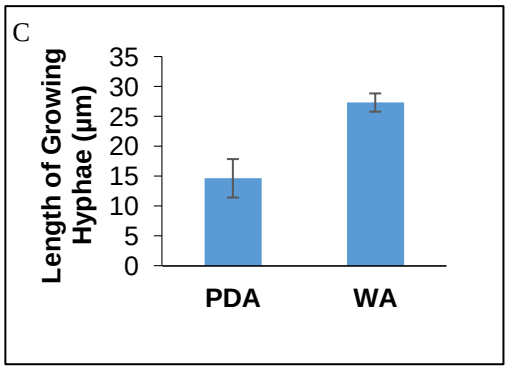

Fig.1 Growth Characteristics of *R. solani* on two different media (basal nutrient medium and nutrient starved medium) (A) emerging hyphae from the inoculum disc at 4 hpi on basal nutrient medium (PDA), (B) hyphae from the inoculum disc at 4 hpi on nutrient starved medium (agar). Bar equivalents to 100µm. (C) Graph represents the differential growth on two different media i.e. PDA and agar

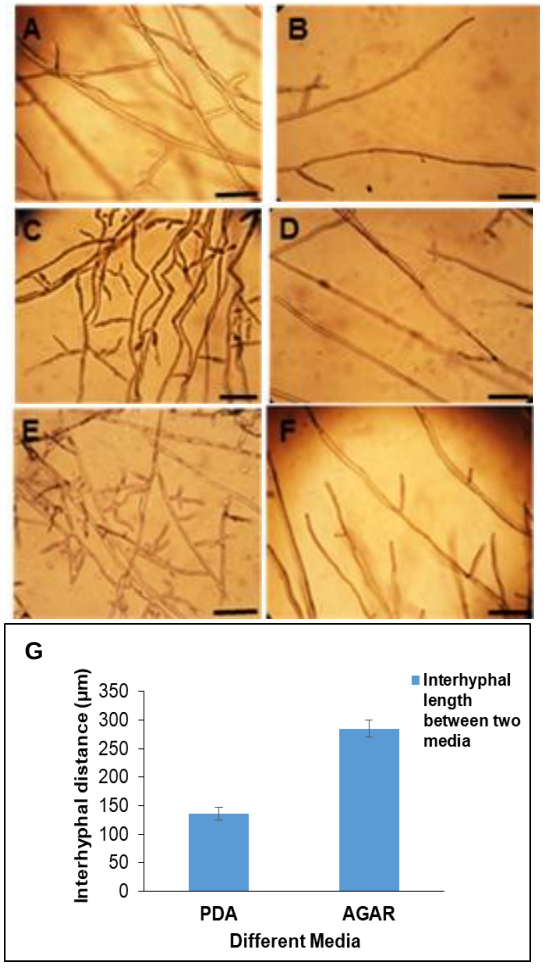

Fig. 2 Comparative account of the intensity of the hyphal mass of the phytopathogen *Rhizoctonia solani* on PDA and agar. (A, B) on 6 hpi, (C, D) on 8 hpi, (E,F) on 24 hpi. Bar equivalents to 100 micrometer. (G) Graph showing comparison of inter-hyphal distance of *R. solani* grown on PDA and agar. Bars represent mean ± S.E.M of three independent experiments with three replicates.

3.2 Development of sclerotium under different conditions

In order to evaluate the consequences of nutrient stress condition on the production of sclerotium, the pathogen was grown in culture condition with two different aforesaid media under complete light and complete dark experimental condition. PDA was found indispensable for the production of sclerotia and favoured by light. On PDA under complete light condition, there were 133±3.6 sclerotia on the plate than complete dark where only 56.33±2.5 sclerotia were produced (Figure 3). In contrast, there was complete absence of sclerotia development on agar under any condition.

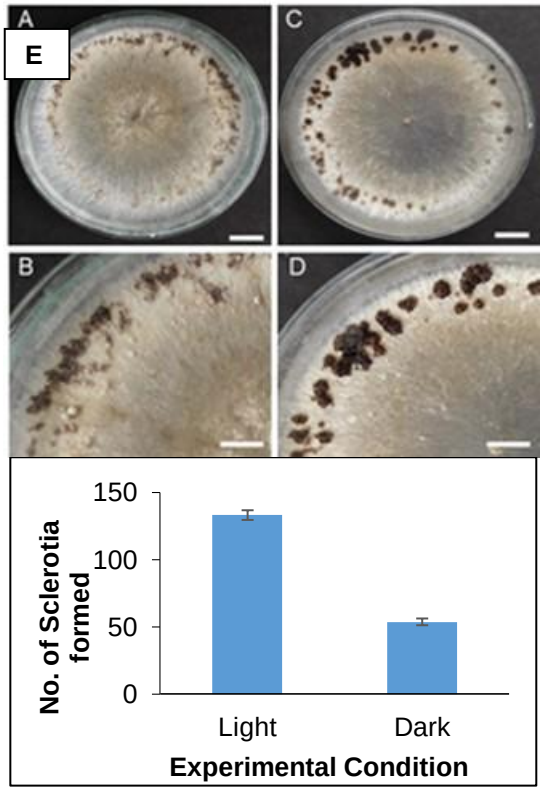

Fig. 3 Variable production of sclerotia under complete light and dark experimental condition. (A) a large number of sclerotia produced on PDA under complete light incubation condition,(B) a magnified view of the picture (A) with sclerotia, (C) less number of clumped sclerotia on PDA medium under complete dark condition, (D) an enlarged view of picture (C). Bar euivalents to 5mm. (E) Graph quantifying the sclerotial production under light and dark condition. Bars represent mean ± S.E.M of three independent experiments with three replicates.

4. Discussion

In the present study, the impact of nutrition on the mycelial growth pattern and sclerotia production under light and dark condition is evaluated. Hyphal growth in filamentous fungi is regulated by a complex network of cellular processes especially by establishing polarity and maintaining that polarity. It always reflects its local environmental condition in its extension rate as well as morphogenesis (Steinberg et al., 2017). Several signature signalling pathway like TOR, HOG etc. globally regulate the response of fungi to nutrient availability (Lin et al., 2015). There is a clear difference in the growth pattern of *R. solani* on PDA basal medium and agar, a nutrient starved medium. In case of PDA, the pathogen grows profusely, with frequent lateral branching, whereas in agar, there is a loose growth with occasional lateral branching. It seems that the nutrient supplemented in the medium supports all the cellular activity needed by the fungus for more branching and more growth. Apical cell length is increased in high CO2 concentration with the reduction in nuclear divisions (Raudaskoski et al., 1982). In *Candida albicans* nutrient limitation, hypoxia or high CO2 concentration upregulate Brg1, while downregulate Nrg1 and also activates Brg1/Hda1 pathway to ultimately stabilize the Ume6 from degradation. This ume6 is essential, for activation of other hyphal genes and to sustain the hyphal elongation (Lu et al., 2013). So, stress condition has its own regulating pathways for maintaining the survival of the organism. The nutrient starved condition in the present study may cause stress to the hyphae which leading to the cell length enlargement, and more distance between two branches. Moreover, on early time points, the hyphae in nutrient stressed medium, intend to be longer than those grown on basal nutrient medium but soon the hyphae paused in its growth in case of agar medium where as it continues to produce more and more hyphae on PDA.

As the necrotrophic pathogen *R. solani* can survive and attack the host both in the mycelium and sclerotial form, it is necessary to know in detail, how these mycelia and sclerotia respond to environmental changes. Differences were clear in the amount of sclerotia production under two different media and under complete light or dark condition. There were almost 3 times more sclerotia produced in case of PDA under complete light experimental condition than in complete dark which was also supported in the work by Mishra et al., 2014. On the contrary, in agar media, there is almost no sclerotial formation in both light and dark condition. Others (Ritchie et al., 2009) also shown that when *R. solani* isolates (AG2-1, AG3) were transferred from nutrient poor to nutrient rich medium, its sclerotial production was highest. The sclerotia grown on the dark, appear darker, and intend to group together. Indeed there is more pigment production occur in response to darkness (Babitha et al., 2008).

5. Conclusions

Conclusively this can be said that, nutrient rich basal medium, enable *Rhizoctonia solani* hyphae to attain a comparative shorter length, thus the two successive branches coming closer. Whereas, the nutrient starved condition impart a longer hyphae with less branches. The sclerotia under continuous illumination becoming numerous, brown in colour. Darkness allow the sclerotia to grow in close proximity, and accumulated more pigmentation thus appearing almost black in colour.

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Reference

[1] Basu A, Chowdhury S, Ray Chaudhuri R, Kundu S. Differential behaviour of sheath blight pathogen *Rhizoctonia solani* in tolerant and susceptible rice varieties before and during infection. Plant Pathology, 65(8): 1333-1346, (2016).

[2] Babitha S, Carvahlo C.J, Soccol R.C, Pandey A, Effect of light on growth, pigment production and culture morphology of *Monascus purpureus* in solid-state fermentation. World Journal of Microbiology and Biotechnology, 24: 2671–2675, (2008).

[3] Bayram O , Krappmann S, Seiler S, Vogt N, Braus G.H, *Neurospora crassa* ve-1 affects asexual conidiation. Fungal Genetics and Biology, 45(2): 127–138, (2008).

[4] Dutta U, Kalha C.S, Srivastava J.N, Effect of different light intensities, different light duration patterns and different temperatures on growth and sclerotial development of *Rhizoctonia solani.* International Journal of Agricultural Sciences, 8(1): 184-18, (2012).

[5] Goswami B.K, Rahaman M.M, Hoque A.K.M.A, Bhuyan K, Mian I.H, Variations in different isolates of *Rhizoctonia solani* based on temperature and pH. Bangladesh Journal of Agricultural Research, 36(3): 389-396, (2011).

[6] Lin X, Alspaugh J.A, Liu H, Harris S.D, Fungal Morphogenesis. Cold Spring Harbor perspectives in medicine, 52: a019679, (2014).

[7] Lu Y, Su C, Solis V.N, Filler G.S, Liu H, Synergistic regulation of hyphal elongation by hypoxia, CO2, and nutrient conditions controls the virulence of *Candida albicans.* Cell Host Microbe, 14(5): 499–509, (2013).

[8] Mishra P.K, Gogoi R, Singh P.K, Rai S.N, Kumar A, Effect of photo Period on morpho-cultural characteristics of *Rhizoctonia solani* f. sp. Sasakii of Maize. Annals of Biology, 30(4): 733-737, (2014).

[9] Nikraftar F, Taheri P, Rastegar M, Tarighi S, Tomato partial resistance to *Rhizoctonia solani* involves antioxidative defense mechanisms. Physiological and Molecular Plant Pathology, 81: 74-83, (2013).

[10] Parissa T, Saeed T, The Role of Pathogenesis-Related Proteins in the Tomato- *Rhizoctonia solani* Interaction. Journal of Botany, 2012: 137037, (2012).
[11] Raudaskoski M, Viitanen H.T, Effect of aeration and light on fruit body induction in *Schizophyllum commune*. Transactions of the British Mycological Society, 78(1): 89-96, (1982).
[12] Riquelme M, Aguirre J, Bartnicki- García S, Braus G.H, Feldbrügge M, Fleig U, Hansberg W, Herrera-Estrella A, Kämper J, Kück U, Mouriño-Pérez R.R, Takeshita N, Fischer R, Fungal morphogenesis, from the polarized growth of hyphae to complex reproduction and infection structures. Microbiology and Molecular Biology Reviews, 82: e00068-17, (2018).
[13] Ritchie F, Bain R.A, McQuilken M.P, Effects of Nutrient Status, Temperature and pH on Mycelial Growth, Sclerotial production and Germination of *Rhizoctonia solani* from Potato Journal of Plant Pathology, 91(3): 589-596, (2009).
[14] Silva M.G, Pozza E.A, Monteiro F.P, Lima C.V.R.V, Effect of light and temperature on *Cercospora coffeicola* and *Coffea arabica* pathosystem. Coffee Science, 11(2): 148-160, (2015).
[15] Singh U. P, Singh S. K, Sugawara K, Srivastava J. S, Sarma B. K, Prithiviraj B, Studies on Sclerotium Formation in Curvularia Species. Mycobiology, 29(3): 154-159, (2001).
[16] Steinberg G, Peñalva M.A, Riquelme M, Wösten H.A, Harris S.D, Cell biology of hyphal growth. Microbiol Spectrum, 5(2), (2017).