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EFFECT OF LIGHT INTENSITY ON MYCELIAL GROWTH AND HYPHAL BRANCHING OF *RHIZOCTONIA SOLANI* KUHN. IN CULTURE

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ABSTRACT: *Rhizoctonia solani* (Kühn) is soil borne necrotrophic pathogen which is found to be sensitive to different environmental conditions. Light intensity plays a crucial role in conferring variation of hyphal growth and branching of this. Hyphal behavior in terms of its growth and branching patterns has been evaluated under different culture conditions. The culturing of the fungus in different light intensities of 120, 20, 5 lux and darkness show that a low light intensity is best for its growth and neither darkness nor high light intensities were preferred. Growth, measured in terms of mycelial spread and hyphal branching, was better in nutrient media rather than nutrient-deficient media. Interestingly even sclerotia formation was reduced when nutrients were not provided.

KEYWORDS: Light intensity, hyphal branching, colony growth, *Rhizoctonia solani*, sclerotium, PDA.

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1.INTRODUCTION

Fungi and their morphogenesis by light induction has been studied earlier, especially in basidiomycetes and zygomycetes members. *Neurospora crassa* being the most well studied model in this field of research. Two important genes *viz*- wc1 and wc2 were revealed to function as photoreceptor for blue light and transcription factor for other light induced phenomenon [1]. It has been reported earlier about the responsiveness of different fungi to light and it ranges from conidiation to sexual development [2, 3]. In *Aspergillus nidulans* illumination promotes sexuality whereas asexual structures were induced when exposed to dark [4]. Starting from fundamental

activity to fine tune regulation of specific mechanisms of daily cellular properties is dependent upon several environmental factors like temperature, pH *etc.* along with light [5]. Hyphal morphogenesis in case of filamentous fungi, are a complex network of cellular processes. Extension of hyphae in filamentous fungi occur by polarity axis establishment and maintenance of this axis [6]. Apolar spores were polarised first to produce new hyphae and this hypha was polarised again during a new branch formation. This process commences at a special hyphal tip complex known as spitzenkorper [7]. Hyphal branches emerging from germ tube of different arbuscular mycorrhizal fungi were induced by light and not only that secondary and tertiary branches were also clustered in response to different combination of light intensities [8]. *Rhizoctonia solani* Kühn is a soil borne, necrotrophic pathogen, having a worldwide distribution sustaining its pathogenic viability both in soil as well as in water in sclerotium form [9]. This sclerotium is formed by coalition of hyphae which originate from repeated branching of short thick lateral hyphae consisting of heavy melanin deposition, high nutrient contents. Later on these clustered hyphae becoming thick walled cells brown to black in colour [10]. This necrotroph is known to cause several destructive diseases by attacking a range of hosts like soybean, potato, sugarbeet, tomato and many few others throughout the world and is capable of producing symptoms including root rot, cankers, damping off, fruit rot, foliage disease [9]. It induces necrosis in hosts and utilizes the necrotic lesions as the initial source of nutrition and as entry points [11]. This fungus being a complex species consisting of several individuals that differ morphologically, physiologically and genetically [9]. This variation also reflects in several parameters like its colony color, growth rate of colony, type of zonation, size and number of sclerotia, saprophytic behaviour, pathogenicity and anastomosing group etc. [12, 13, 14]. It has been classified in 14 anastomosing group (AG) till date, namely AG 1 to 13 and one Bridging Isolate (BI) [15]. It was reported by Lokesha and Somashekar (1988) that sclerotia of *R. solani* grow faster with high number of sclerotia production occurred under visible light than UV light and darkness [16]. Different isolates of *R. solani* and the optimum pH and temperature were also evaluated by many workers [12, 13]. But there is merely any report which directly evaluated the variation of different orders of branching against different light intensities on *R. solani*. In the present study, effect of light on *R. solani* is analysed in terms of mycelial growth, mycelial branching pattern in respect of three different light intensity and complete dark experimental condition in two different media. *R. solani*, as being a filamentous plant pathogenic fungus, impart its response by changing its branching behaviour. The principal structural part of all the filamentous fungus is thread like hyphae which vary in terms of septation, shapes and nuclear content per hyphae [17, 18]. It is reported earlier that low light intensity favours more mycelial growth [10]. So, it is important to study the differential response of fungus to light and dark especially of those causing disease to crop plant as it helps to reveal the factors regulating the pathogenicity of the fungus.

2. MATERIALS AND METHODS

Fungal Material

Rhizoctonia solani Kühn. (AG1-1A isolate, by Rice Research Station, Chinsurah, West Bengal, India) was maintained as a pure culture by growing it on Potato Dextrose Agar (PDA) medium at 28°C, and sub-cultured once in a month, maintaining the previous growth conditions. This was used in this study as the pathogen.

Preparation of Inoculum

To perform this experiment, a 3mm mycelial disc was utilized as the inoculum. Mycelial discs were obtained from the growing edges of the 5-day-old culture of *R. solani* with the help of a sterile cork borer.

Study of the differential colony growth of *R. solani* on two different media

PDA and agar plates were made and inoculated with a 3mm mycelial disc as already stated. Mycelial growth from the inoculum disc was observed and compared in case of each media at 24 hours post inoculation (hpi).

Comparison of hyphal branching under three different light intensities and dark condition

In order to observe the variable response of *R. solani* under different light intensities, as well as under complete dark condition, PDA and agar plates were made aseptically. These plates were inoculated as above and were incubated at three different light intensities (high-120 lux, medium-20 lux and low 5 lux) and dark. Adequate moisture content was maintained by keeping the plates on the wet bed of moist tissue paper. Different orders of branching (1st, 2nd, 3rd) were observed at different observation points i.e. 0hr, 2hr, 4hr, 6hr, 8hr, and 24hr under a compound microscope (Leica DMLS, 20x-100x) according to a standardized protocol [19].

Statistical analysis

All data were analysed using standard statistical method. Data are mean of three independent experiments ± S.E.M with three replicates.

3. RESULTS AND DISCUSSION

Mycelial Growth on two media with different nutritional constituents

PDA (basal nutrient media) and agar (without nutrition) plates that were inoculated with 3mm in diameter mycelial plug of *R. solani*, were observed for their growth at 24-hour post inoculation under three different light intensities as well as under complete dark condition. It was found that growth was increased with decreasing light intensities (Fig. 1 A-H) and highest mycelial growth in respect of colony diameter, was observed under lowest light intensity of this experiment (Fig. 1 E, F). In contrast, under complete dark condition (Fig. 1 G, H), the growth is reduced than the lowest light intensity (Fig. 1 E, F), but higher than the other two intensities (Fig. 1 A-D). In addition, as in all cases, the hyphae grow more profusely in case of PDA (Fig1 A, C, E, G) than the agar, (Fig. 1 B, D, F, H).

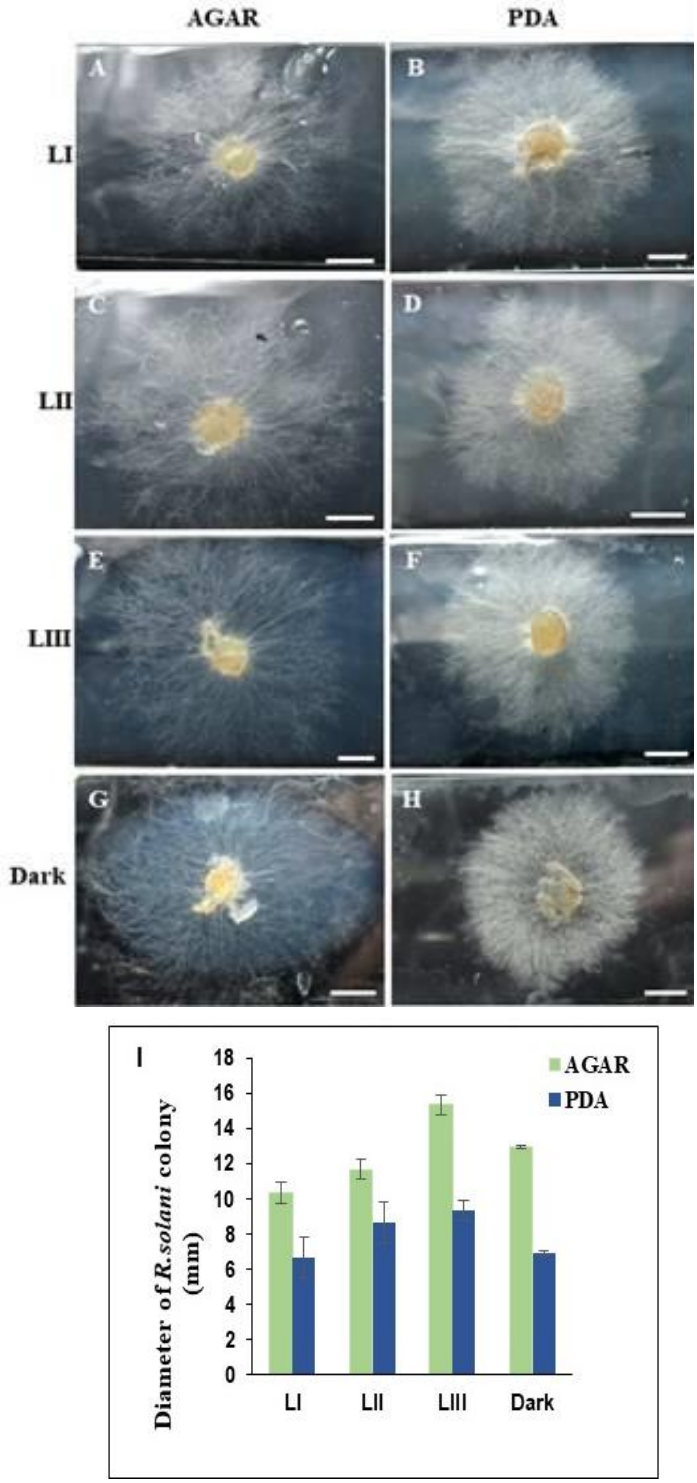
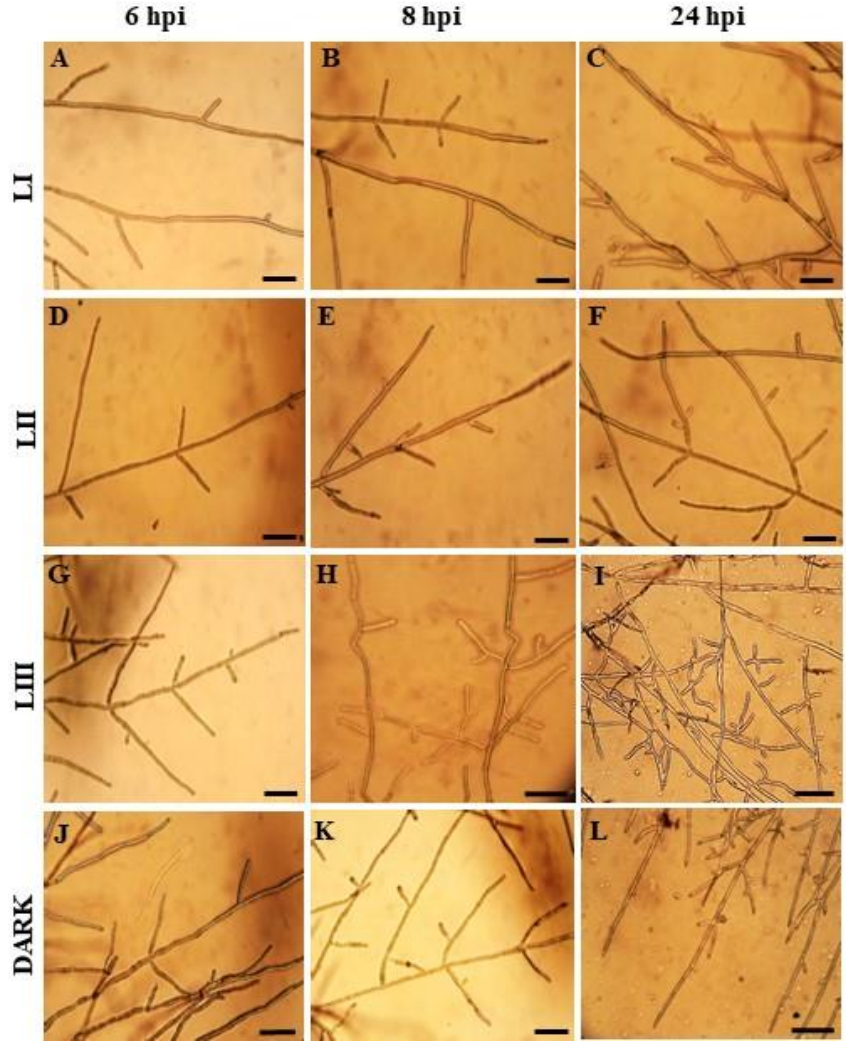


Fig. 1 Comparison of mycelial growth from inoculum disc at different light intensities (LI, LII, LIII) and dark condition at 24 hours post inoculation (hpi) on two different media- potato dextrose agar (PDA) & agar. (A,B) under high (120 lux) light intensity(LI); (C,D) under medium (20 lux) light intensity(LII); (E,F) under low (5 lux) light intensity (LIII); (G,H) under complete dark condition. Bar equivalents to 5 mm. (I) Graph representing the differential colony growth under 3 different light intensities and dark condition on 2 different media. Bars represent mean ± S.E.M of three independent experiments with three replicates.

Branching Pattern under different light intensity

The hyphal branching pattern was analysed on PDA and on agar under three different light intensities and dark condition. Observations were made at 0, 2, 4, 6, 8, 24-hour time points. Results revealed that there was no emergence of hyphae up to 2-hour in both PDA and agar. From 4-hour time point, hyphae start to radiate from the inoculum disc but without the formation of lateral branches, and 6-hour onwards, there was branching within the mycelium in order to anastomose with each other. It was found that, the branching number as well as different levels of branching order, i.e. 1st order, 2nd order and 3rd order branching is increased as the light intensity decreases, and the time period pass on (Fig. 2 A-L). In case of the highest light intensity at 24 hpi, only 1st order branching is seen (Fig. 2 C) compare to the medium and lowest light intensities where both 1st and 2nd and all the three orders of branching is appeared respectively (Fig. 2 F, I). Maximum number of branching is found in lowest light intensity at 24 hpi (Fig. 2 I). There is a significant reduction of branches of all order on agar only than on PDA (Fig. 3 A-L), as evidenced by the graphs also (Fig. 2 M, N, O and Fig. 3 M, N, O).



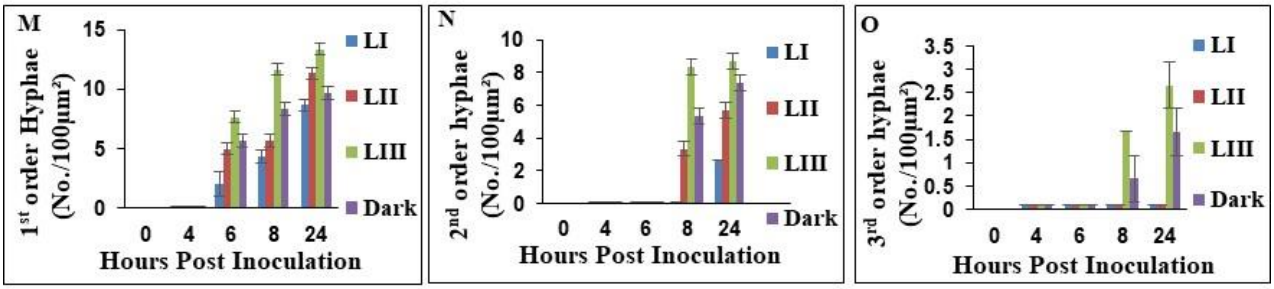


Fig. 2 Microscopic observation of different branching order in the phytopathogen *Rhizoctonia solani* in response to 3 different light intensities and complete dark condition on potato dextrose agar (PDA). (A, B, C) shows the branching pattern under high (120 lux) light intensity (LI) at 6, 8, and 24 hpi respectively. (D, E, F) represents the branching incidence under medium (20 lux) light intensity (LII) with same observation point. (G, H, I) stands for branching pattern under low (5 lux) light intensity (LIII) with same observation points, and finally (J, K, L) shows the same under complete dark condition with same observation points. Bar equivalents to 100 micrometre. (M) Comparison of 1st order branching under 3 different light intensities (LI, LII, LIII) and dark condition. (N) Comparison of 2nd order branching under above said experimental condition. (O) Comparison of 3rd order branching under the same experimental condition as above. Bars represent mean ± S.E.M of three independent experiments with three replicates.

Branching Pattern under dark condition

Branching pattern was also evaluated under complete darkness. The branching incidence was found to be directly proportional with increasing time as evidenced by increasing number and presence of branches of all orders at 24 hpi than the other two time points (Fig. 2 J, K, L and Fig. 3 J, K, L). In addition, under dark condition 1st, 2nd, and 3rd order of branching number is significantly lower than the lowest light intensity of this experiment (Fig. 2 G, H, I). There is complete lack of 3rd order branching in case of lowest light intensity as well as in complete dark condition at 24 hpi on the agar media, as compared to PDA (Fig. 3 J, K, L).

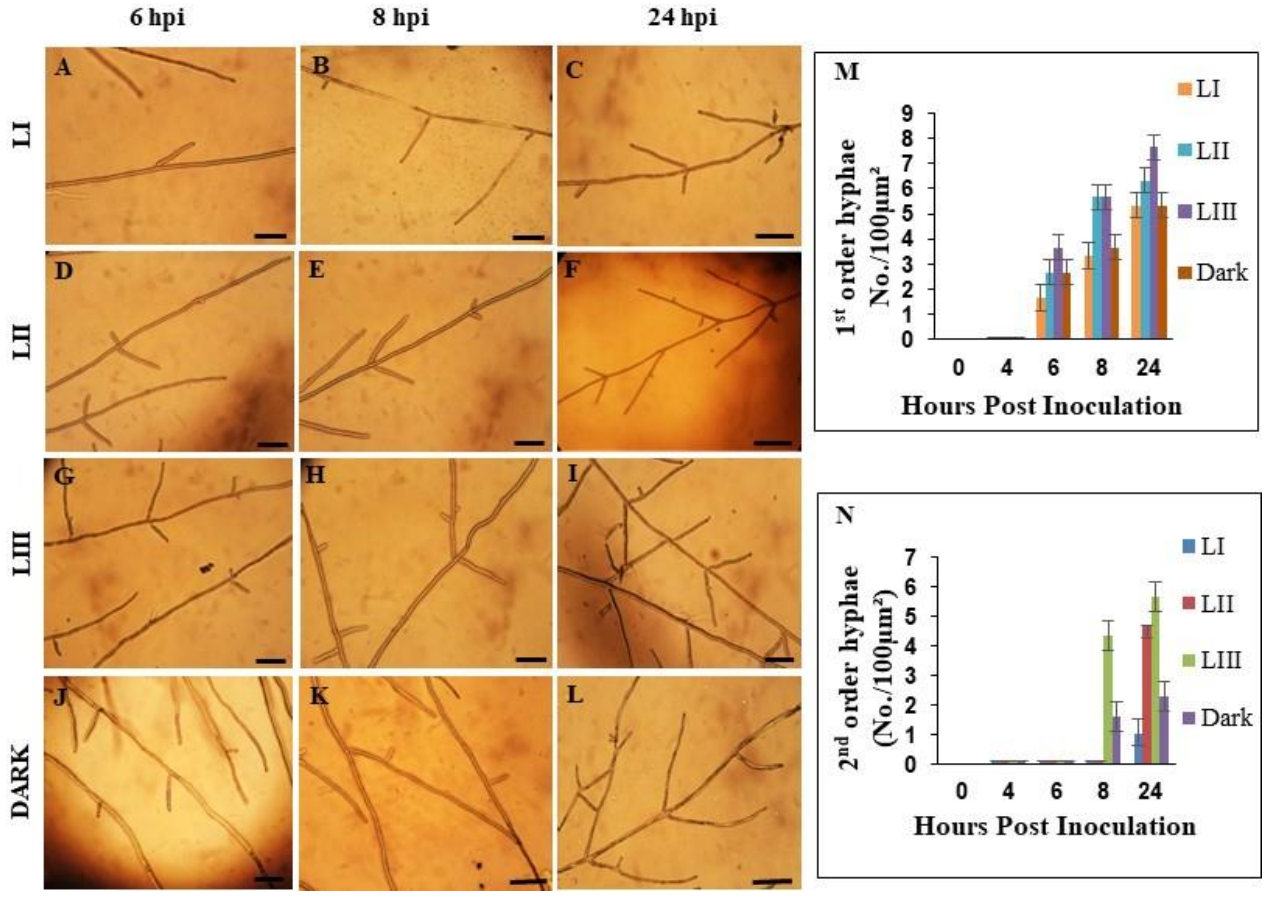


Fig. 3 Microscopic observation of different branching order of *Rhizoctonia solani* in response to 3 different light intensities and complete Dark condition on agar medium. (A, B, C) represents the hyphal branching under high (120 lux) light intensity at 6, 8, 24 hpi respectively. (D, E, F) showing the same under medium (20 lux) light intensity at the above said time points. (G, H, I) Shows the branching pattern under low (5 lux) light intensity at the same time points and (J, K, L) stands for the same incidence under dark experimental condition over 6, 8, 24 hpi respectively. (M) Comparison of the 1st order branching under 3 different light intensities (LI, LII, LIII) at same time points. (N) Represents 2nd order branching with same parameters. Bars represent mean ± S.E.M of three independent experiments with three replicates.

DISCUSSION

Light induced branching was recorded earlier in different arbuscular mycorrhizal fungi [8]. In this report, maximum colony diameter of *R. solani* was found under low light intensity on agar media, followed by dark condition, medium and high light intensity respectively. Similar facts were also shown by Dutta and workers [10] where radial colony growth as well as sclerotia production were highest at low light intensity. Though the hyphal length of the mycelia of *R. solani* remain longer on agar media but the overall growth was much more profuse under PDA over agar. Not only the mycelial growth, but also the sclerotium production was found to be highest for PDA (data not shown). This incident may vary over different isolates of *R. solani* as shown by Ritchi and workers.

[20]. The branching pattern and its order also followed the same trend. More intense branching with all the three-branching orders viz. 1st, 2nd, and 3rd was found at lowest light intensity (5 lux) at 24 hpi on PDA. On agar, 3rd order branching was completely absent. It was reported that branching pattern of *Curvularia affinis* was also increased on PDA than other media [21]. Indeed, several fungus enhance branch formation under ameliorative condition whereas, under stressed condition like, high CO_2 concentration, oxygen limitation etc. they induce hyphal elongation than lateral branching [22, 23. 24, 25]. Along with branching, different quality of light also regulate the fruit body and pigment production in many fungi [26, 27, 28, 29]. Blue light is essential for maintaining the asexual reproduction and survival in absence of carbon source in *Trichoderma* [30]. In some mycorrhiza specific strigolactones are known to induce more branching [31]. Hence the above facts revealed that light along with all other essential components are supportive for more branching as well as intense growth and asexual reproduction also. In this study, more cellular energy and lots of proteins which the fungus get from the nutrient rich medium are the driving force behind more branching whereas nutrient starved medium did not allows much activity in terms of branching.

4. CONCLUSION

It can be concluded from the above study that, light induces hyphal branching in *Rhizoctonia solani* but at a specific intensity. The pathogen can maintain its survival in dark condition also. Different media impart different effect on hyphal branching as well as on vegetative growth. This study is significant in understanding the differential behaviour of the pathogen under different light intensity and media composition, in order to study further the host pathogen relationship and disease development.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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