Bot. Bull. Academia Sinica 18: 39-44 (1977)

LIGHT INHIBITS SCLEROTIAL FORMATION OF AN ISOLATE OF *HELMINTHOSPORIUM SIGMOIDEUM* WHICH CAUSES STEM ROT OF *ZIZANIA LATIFOLIA*[1,2]

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(Received April 14, 1976; Accepted October 26, 1976)

Abstract

A pathogenic fungus causes stem rot on *Zizania latifolia* is identical to *Helminthosporium sigmoideum*, the causal organism of rice stem rot, in spore and sclerotial morphology. The main differences in these two isolates are that *Z. latifolia* isolate is not pathogenic to rice and it sporulated well on V-8 juice agar and *Oryza sativum* isolate sporulated only when it was grown on water agar or floating its sclerotia on water. The conidial formation of *Z. latifolia* isolate was light dependent and was only induced by near ultraviolet (NUV) radiation. However, its sclerotial formation was inhibited by light particulary NUV radiculation.

Introduction

Helminthosporium sigmoideum Car. and *H. sigmoideum* var. *irregulare* are the causal orgamisms of stem rot of rice, a severe disease which has been widely studied (Ou, 1972). A similar pathogenic fungus, which causes stem rot on *Zizania latifolia*, has also been named *H. sigmoideum*. Morphologically, these two isolates are almost identical, though Hsieh and Liang (1975) argued that the number of septa on few conidia of *H. sigmoideum* isolated from *Z. latifolia* was four instead of three as usually found on the isolate on *Oryza sativa*. According to our observations, under some unknown conditions, there were few conidia with four septa but were rare, and we considered that this fact was not important from taxonomical point of view. However, there were distinct differences existing between these two isolates, i.e., pathogenicity and sporulating characteristic. Hsieh and Liang failed to obtain positive

(1) Paper No. 185 of the Scientific Journal Series, Institute of Botany, Academia Sinica.
(2) This study was financially supported by the National Science Council and Joint Commission on Rural Reconstruction, Republic of China.

inoculation of *Zizania latifolia* isolate to *Oryza sativa* and we have obtained the same result. *Z. latifolia* isolate produced abundant conidia on V-8 juice agar medium under light, whereas *Oryza sativa* isolate failed to produce conidia under the same conditions and produced them only by floating the sclerotia on water or placing the sclerotia on water agar surface (Luttrell, 1963). In preliminary observations we found that light has profound effect on sporulation and sclerotial formation of *H. sigmoideum* of *Z. latifolia* isolate. In this report we present the results of our attempt to clarify some effects of light quality on sporulation and sclerotial formation of this fungus.

Materials and Methods

Sources of test fungal isolates: The isolates of *Helminthosporium sigmoideum* were isolated from *Oryza sativa, Zizania latifolia* and *Z. aquatica*, and they were designated as *O. sativa* isolate, *Z. latifolia* isolate and *Z. aguatica* isolate of *H. sigmoideum* respectively in this report. The last isolate was supplied by Mr. Robert Kroll of the Department of Plant Pathology, University of Minnesola. The *O. sativa* isolate was cultured on potato sucrose agar slant as stock culture and the other two on V-8 juice agar slant as stock cultures. V-8 juice agar medium was used for studying the sporulation and sclerotial formation throughout the experiments.

Source of light: A 20-watt fluorescent lamp and a 20-watt black light lamp were used as light sources. The fluorescent lamp provided wide spectrum of wavelengths of radiation and black light lamp provided mainly the near ultraviolet (NUV). Different wavelengths of radiation were obtained by transmitting fluorescent light (emitted from fluorescent lamp) through different Corning glass filters. The glass nos. are 4602 (bluish, infrared absorbing, visible transmitting), 3850 (clear, NUV transmitting), 5850 (purple), 5543 (blue), 4305 (blue-green), 4010 (green), 3486 (yellow), 3391 (straw), and 2402 (red). The light sources were set 25 cm above the petri-plates. The experiments were carried out at 25° to 28°C. The light intensity was 1,000 foot candle from fluorescent light lamp and 200 foot candle from black light lamp.

Results

Conidial production and sclerotial formation of Z. latifolia isolate on different media

Water agar, V-8 juice agar, carrot agar, yeast extract agar, potato sucrose agar (PSA) and potato glucose agar (PDA) were used. Cultures tested for conidial production were placed under light and for sclerotial formation at darkness. One week after incubation only those cultures grown on water agar,

V-8 juice agar and carrot agar produced conidia, among them V-8 juice agar was the best one for conidial production (Table 1). However, this isolate formed sclerotia only on V-8 juice agar and carrot agar among tested media used.

In considering the results shown in Table 1, we suspected that sucrose and glucose in PSA and PDA might have inhibitory effect on conidial formation. With this speculation in mind, we carried out the following experiment: two percent and 4 percent sucrose and glucose were added to V-8 juice agar medium respectively, and then *H. latifolia* isolate of *H. sigmoideum* was inoculated on to them. V-8 juice agar without adding sugar was served as control. One set of the cultures was incubated under light conditions and the another set was at darkness. One week after incubation all culture grown on V-8 juice agar with and without glucose or sucrose under illumination produced abundant conidia and those grown at darkness did not. Meanwhile the sclerotia were produced only at darkness. The result revealed that sucrose and glucose did not inhibit sporulation of this fungus.

Table 1. *Sporulation and sclerotial formation of* ***Zizania latifolia*** *isolate of* ***Helminthosporium sigmoideum.***

Medium	Sporulation	Sclerotial formation
Water agar	+	+
V-8 juice agar	+	+
Carrot agar	−	+
Yeast extract agar	−	−
PSA	−	−
PDA	−	−

+, with sporulation or sclerotial formation;
−, without sporulation or sclerotial formation.

We also suspected that components of potato decoction might have inhibitory effect on the sporulation. However, by culturing it on the medium with 3 parts of V-8 juice and 1 part of potato decoction, this isolate sporulated well on this mixed medium as it did on plain V-8 juice agar medium. This result reject the speculation that potato decoction might contain some components which inhibited conidial formation. So far we can not explain the failure of this fungus to sporulate on PSA and PDA media.

Effect of light on sporulation and sclerotial formation of three isolates of H. sigmoideum

Oryza sativa isolate, *Z. latifolia* isolate and *Z. aquatica* isolate of *H.*

sigmoideum were grown on V-8 juice agar medium. One set of cultures was placed under light and the another set was placed at darkness. Ten days after incubation the *O. sativa* isolate produced no conidia neither at darkness nor at light, whereas it formed sclerotia under both conditions. *Z. latifolia* isolate and *Z. aquatica* isolate, on the contrary, sporulated only under light conditions and light apparently inhibited sclerotial formation. Zonation on colonies due to the distribution of conidial formation appeared when the cultures were incubated under alternating light and darkness daily (Table 2).

Table 2. *Effect of light on sclerotial formation and sporulation of three* ***Helminthosporium sigmoideum*** *isolates*

Isolate	Sclerotial formation		Sporulation	
	Light	Dark	Light	Dark
Zizania latifolia isolate	−	+	+	−
Zizania aquatica isolate	−	+	+	−
Oryza sativum isolate	+	+	−	−

+, with sporulation or sclerotial formation;
−, without sporulation sclerotial formation.

Light quality effect on the conidial and sclerotial formation of Z. latifolia isolate of H. sigmoideum

The cultures were grown on V-8 juice agar medium under continuous irradiation of different wavelength of radiation for 10 days. The results in Table 3 show that only the wavelength of radiation in the region of near ultraviolet (NUV) (obtained from Corning glass filters: glass nos. 4602 and 5850) possesses the inhibitory effect on sclerotical formation. The wavelengths in the regions of blue, green, yellow and red did not show inhibition to the sclerotial formation. These sclerotia developed on the cultural plates covered by Corning glass filters nos. 4602 and 5850 were formed underneath the agar but not on the surface of agar plate. Thus it appears that the wavelengths of radiation passing these two glass filters probably do inhibit sclerotial formation. The sclerotia formed underneath the agar might be due to the elimination of active wavelengths inhibitory to sclerotial formation by agar. We made another experiment to further verify our speculation just mentioned. The inoculation of the fungus was made by placing the inoculum on top of sterilized dialyzing membrane which was placed on the surface of agar medium instead of directly placing the inoculum on the surface of agar medium. Ten days after incubation under continuous illumination of different wavelengths of radiation, no sclerotia formed on the surface of dialyzing membrane which were exposed to NUV.

Table 3. *Effect of light quality on sclerotial formation and sporulation of* ***Zizania latifolia*** *isolate of* ***Helminthosporium sigmoideum***

Corning glass no.	Color description	Number of sclerotia*	Sporulation
4602	bluish, infrared absorbing, visible transmitting	8	+
5850	purple	13	++++
4305	blue-green	39	—
5543	blue	50	—
4010	green	46	—
3486	yellow	41	—
3391	straw	49	—
2418	red	52	—
2402	red	48	—
Dark		48	—
NUV		2	++++

++++, abundant conidia were preduced; +, few conidia were produced.
*, in a circular area with diameter 0.5 cm.

Disscussion

Light effects on sclerotial formation so far have reported only in a few groups of fungi. Light either induces or inhibites sclerotial formation. There is no correlation between phylogenic relation of the fungi and their response to the effect of radiation. Our results showed that *H. sigmoideum* of *O. sativa* isolate produced sclerotial either at light or at darkness, whereas the sclerotial formation of *Z. latifolia* isolate was inhibited by light in the region of NUV. Blue, green, yellow and red light did not inhibit sclerotial formation of this fungus. The ineffectiveness of the blue light to inhibit sclerotial formation of present test fungus was surprising since blue light has been reported as the most effective region of radiation to inhibit sclerotial formation of *Verticillium alboatrum* (Kaiser, 1964) and *Botrytis cinerea* (Tan and Epton, 1973). The complete inhibition of sclerotial formation by near ultraviolet radiation (peak 365 nm) has only been documented once with an isolate of *Verticillium dahliae* (Brandt, 1964).

Conidial production of *H. sigmoideum* of the three isolates were absolutely light dependent. The cultures grown at darkness produced no conidia but sclerotia only. Furthermore, only NUV radiation was effective in inducing sporulation. The photomorphogenic reaction was same as happened in many fungi classified in Fungi Imperfecti (Leach, 1971; Honda, 1969 and Chang, 1974) such as *Helminthosporium oryzae*, *Alternaria solani*, and *Aschochyta pisi*. Their sporulation was initiated by blue light and NUV radiation particularly

the latter one. One step of conidiation was different between present test fungus and three fungi just mentioned. *Z. latifolia* isolate of *H. sigmoideum* is not a 'diurnal sporulator', it completed its conidiation under continuous illumination.

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光線對茭白莖腐病菌 *Helminthosporium sigmoideum* 菌核形成之抑制

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茭白莖腐病菌 *Helminthosporium sigmoideum* 培養在 V-8 汁培養基上形成大量的分生胞子和菌核。分生胞子和菌核的形成，系受光線的節制；經光線照射本菌才會誘發分生胞子形成，同樣情況下本菌不形成菌核，菌核只在無光照下才形成。而且只有近紫外光 (near ultraviolet light) 才能誘發分生胞子之形成和抑制菌核之形成。