IDENTIFICATION OF CAUSAL FUNGI OF *SCLEROTINIA DISEASE* OF LEGUMES IN THE CERRADOS

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Introduction

White mold of legumes is an important disease in the Cerrado region of Brazil (1,2,3). Irrigation in the dry season and monoculture of susceptible crops increase the inoculum potential of pathogens. Sclerotinia sclerotiorum (Lib.) de Bary and Sclerotinia trifoliorum Erikss had been known to be the causal fungi of Sclerotinia stem rot of legumes and differ in their epidemiological characteristics (4,5). Therefore accurate identification is necessary to control the disease. The objective of this study was to differentiate the isolates of Sclerotinia sp. collected in the Cerrados with Sclerotinia sclerotiorum and Sclerotinia trifoliorum imported from Japan.

Materials and Methods

Fungus: Fourteen isolates of Sclerotinia sp. obtained by Dr. Mitsueda in 3 areas of the Cerrado region in Brazil were used (Table 1). Standard isolates of Sclerotinia sclerotiorum (SB-1) and Sclerotinia trifoliorum (YW-4) were imported from Japan (import permits Nº 03782).

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TABLE 1 - Location, year of isolate collection and source plants of *Sclerotinia* spp.

Isolate	Location	Year	Source plant
S-B1[a]	Morioka, Japan	'89.7	Rape
YW-4[b]	Yamagata, Japan	'89.4	White clover
YA-1	Morioka, Japan	'90.4	Alfalfa
Rond	Brasília DF, Brazil	'90.7	Common bean
Suika	Paracatu MG, Brazil	'91.8	Watermelon
Kosyo	Brasília DF, Brazil	'90.8	Pepper
Tomato	Brasília DF, Brazil	'91.7	Tomato
Endo	Brasília DF, Brazil	'90.8	Pea
Claud	Brasília DF, Brazil	'90.7	Common bean
SG-1	São Gotardo MG, Brazil	'91.3	Soybean
SG-2	São Gotardo MG, Brazil	'91.3	Soybean
SG-3	São Gotardo MG, Brazil	'91.3	Soybean
P-1	Paracatu MG, Brazil	'91.2	Common bean
P-2	Paracatu MG, Brazil	'91.2	Common bean
P-3	Paracatu MG, Brazil	'91.2	Commom bean
P-4	Paracatu MG, Brazil	'91.2	Common bean

a) standard isolate of *Sclerotinia sclerotiorum* identified in Japan.
b) standard isolate of *Sclerotinia trifoliorum* identified in Japan.

Cultural properties: The mycelial growth rate of Sclerotinia sp. was determined by placing each 9 mm mycelial disc on a PDA plate incubated at 5, 10, 15, 20, 25, 27, 30°C and by measuring the mycelial length every 24 hours. Two standard isolates of *Sclerotinia sclerotiorum* (SB-1) and *Sclerotinia trifoliorum* (YW-4) were used for comparison with the characteristics of the present isolates of *Sclerotinia* sp. collected in Brazil.

Mycelial interactions between Sclerotinia sp. and the standard isolates were tested by placing each isolate on a Difco- PDA plate in a 9 cm Petri dish at 20°C for two weeks. Each 9 mm agar disc from the margins of actively expanding young colonies on PDA was plated 5-6 cm apart in each dish with three replications. Mycelial interactions in the regions of association between the isolates were examined according to the three reaction types defined by Wong and Willets (6): (i) free intermingling of mycelia without antagonism and occasional hyphal anastomoses; (ii) formation of a raised white zone between mycelia, later becoming darkly pigmented, this incompatibility zone associated with excessive branching of hyphal tips at the margins of one or both of the colonies; (iii) brown incompatibility zone between mycelia, accompanied by early lysis of the tips of the peripheral hyphae of one of the colonies.

Results and Discussion

Relationship between mycelial growth and incubation temperature: Temperature responses of mycelial growth of Sclerotinia sp. collected in the Cerrados on PDA were similar to those of S.sclerotiorum SB-1 but not to those of S. trifoliorum YW-4 (Fig.1). Optimal temperature for mycelial growth of Sclerotinia sp. ranged from 20°C to 27°C, while very little growth occurred at 30°C and 5°C. The S. trifoliorum YW-4 grew more slowly than Sclerotinia sp. and its optimal temperature was lower than 20°C. A brown pigmentation was observed in the cultures of Sclerotinia sp. at a temperature above 25°C during the 10 day period of incubation. Optimal temperature for sclerotial formation of Sclerotinia sp. ranged from 20°C to 25°C, while sclerotia were not formed at 30°C and below 10°C.

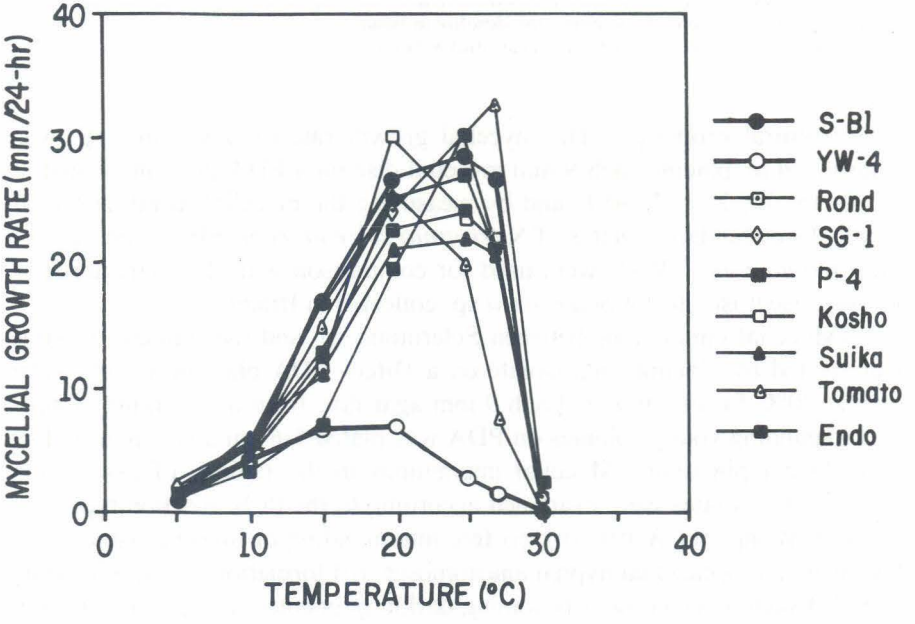

FIG. 1 - Effect of temperature on mycelial growth rate of *Sclerotinia* sp. collected in Brazil and standard isolate of *Sclerotinia sclerotiorum* (SB-1) and *S. trifoliorum* (YW-4).

Mycelial interaction:

The mycelial interaction between *Sclerotinia* sp. from the Cerrados and *S. sclerotiorum* SB-1 or *S. trifoliorum* YW-4 was studied. The mycelia of the isolates from the Cerrados and those of *S. sclerotiorum* SB-1 intermingled freely without the formation of a brown zone in the regions of association on PDA medium, and they corresponded to Wong's (6) reaction type (i). On the other hand, a white zone could be seen without magnification between the mycelia of the isolates from the Cerrados and those of *S. trifoliorum* YW-4 (Table 2). Microscopic examination revealed that this white zone was due to the excessive branching of hyphal tips of one or both colonies (Fig 2). This type of interaction corresponded to Wong's (6) reaction type (ii). A dark brown pigmented zone along the region of association was observed between *S. sclerotiorum* SB-1 and *S. trifoliorum* YW-4. On the other hand, a pigmented zone was not clear by revealed between the Cerrado isolates and *S. trifoliorum* YW-4 (Fig.3). This difference in pigmentation may indicate the presence of geographic variations in the physiological metabolism of the isolates of *S. sclerotiorum* between Japan and Brazil.

TABLE 2 - Types of mycelial interaction occurring between isolates of *Sclerotinia* sp. collected in Brazil, *S. sclerotiorum* and *S. trifoliorum* 10 days after inoculation on PDA at 20 °C.

Isolate	Reaction type[a]	
	YW-4	SB-1
YW-4	(i)	(ii p+)
SB-1	(ii p+)	(i)
Rond	(ii p-)	(i)
Suika	(ii p-)	(i)
Kosyo	(ii p-)	(i)
Tomato	(ii p-)	(i)
Endo	(ii p-)	(i)
Claud	(ii p-)	(i)
SG-1	(ii p-)	(i)
SG-2	(ii p-)	(i)
SG-3	(ii p-)	(i)
P-1	(ii p-)	(i)
P-2	(ii p-)	(i)
P-3	(ii p-)	(i)
P-4	(ii p-)	(i)

[a] Data based on modified Wong and Willets' mycelial reaction type: (i) mycelia intermingle freely without any incompatible reactions
(ii p+) formation of a white zone between mycelia, later becoming darkly pigmented.
(ii p-) formation of a white zone between mycelia without pigmentation.

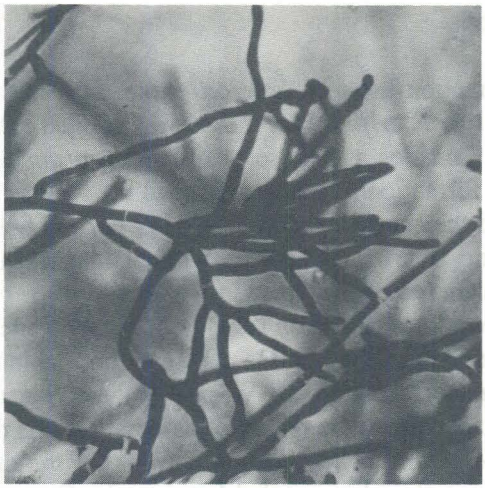

FIG. 2 - Execessive hyphal branching reaction of SG- 1 when approaching the mycelial front of YW-4 in PDA agar.

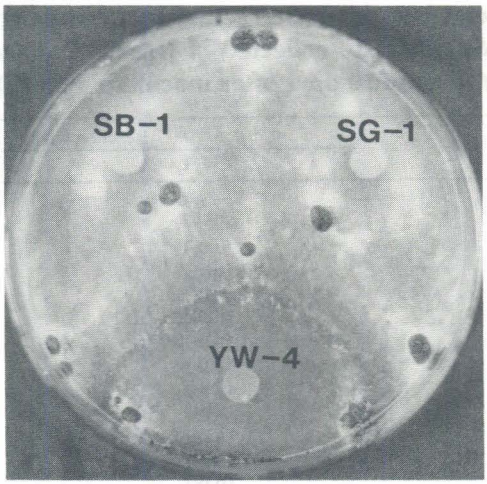

FIG. 3 - Mycelial interaction among three isolates of *Sclerotinia*. Reaction between SB-1 and SG-1 showing no antagonism, between SB-1 and YW-4 showing brown pigmentation, between SG-1 and YW-4 showing a white reaction zone.

Co-inoculation method could be useful for practical application in specific identification, if standard identified isolates are available. The reaction type between mycelia of the isolates reflects their genetic relationship. The intermingling of hyphae without incompatibility and anastomosis in type (i) suggests a very close relationship. The white zone due to excessive branching of hyphal tips in type (ii) indicates a genetic incompatibility between the Cerrado isolates and *S. trifoliorum*.

In conclusion, all the isolates of *Sclerotinia* sp. collected in the Cerrado region should be identified as *Sclerotinia sclerotiorum*. However, the determination of the number of nuclei in the ascospore is necessary for final identification.

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