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RELATIONSHIP AMONG *GANODERMA* SPECIES AFFECTING ARECANUT AND COCONUT PALMS

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ABSTRACT

Though *Ganoderma lucidum* Leys (Karst) was reported to cause *Anabe* disease in coconut and arecanut palms, other species like *G. boninense* and *G. applanatum* are also known to affect these palms. The studies indicated that both *G. lucidum* and *G. applanatum* could cause infection in coconut and arecanut palms and both are cross inoculable. The gel diffusion tests also indicate a fairly high degree of affinity among these two species. *Ganoderma* species affecting arecanut and coconut palms in the maidan areas of Karnataka are inter-related pathotypes.

INTRODUCTION

Although *Ganoderma lucidum* Leys (Karst) was reported to cause Anabe disease in coconut and arecanut palms (Venkatarayan, 1936), other species like *G. boninense* and *G. applanatum* are also known to affect coconut (Peries, 1974; Bhaskaran *et al.* 1989). However, these species have not been recorded so far in Karnataka where a large number of arecanut and coconut palms are affected by *Ganoderma* root rot. According to Furtado (1965) basidiospore morphology is the most dependable taxonomic character among Ganodermoideae. Since the morphological studies alone may not be able to bring about the variability clearly, studies on serological relationship are also necessary to study the relationship among isolates.

In order to study the species variation and their reaction to coconut and arecanut palms, the present investigations were taken up.

MATERIALS AND METHODS

Morphological studies of basidiocarps and basidiospores of both coconut and arecanut isolates of *Ganoderma*, were made from the specimens collected in nature. Both the isolates were used to study their cross infectivity. *Ganoderma* affected arecanut stumps were planted in the basins of healthy coconut palms and *vice versa* taking care to see that the roots of diseased stump are in contact with the roots of healthy palms. Ten palms each were inoculated adopting this method.

The cultures of *Ganoderma* used in the serology studies were isolated from fresh basidiocarp and were grown for 30 days in Waksman liquid medium in still condition at room temperature (28± 2°C). The mycelium was filtered, dried and powdered. 600-700 mg of mycelium was ground in 15-20 ml physiological saline (PBS) and filtered through Seitz filter. The filtrate was made upto 20 ml and used as antigen. Antiserum was produced by immunizing the rabbits with intramuscular injections (1 ml of antigen with equal volume of Freund's complete adjuvant) thrice a week. The rabbits were bled and the antisera collected. A total of six antisera were produced against the respective six antigens from arecanut - Agd-H (Hirehalli), Agd-V (Vittal), Coconut Cgd-K (Kadur), Cyd-H (Hirehalli) and Cyd-A (Arsikere) and one from Acacia - dd (Dehradun).

The serological relationship among the six isolates was studied by tube agglutination tests and agar gel diffusion plates.

Agar gel diffusion test

Agar gel diffusion plates were prepared with the desired number of wells into which the

* CPCRI, Kasaragod 671 124

reactants were placed. The antigens were placed in the pheripheral wells while the antisera in the central one using sterile pipettes. Each well contained 0.1 ml of the reactant liquid. Plates were incubated at 25°C under humid conditions and observations were made at regular intervals.

Agglutination test

0.5 ml of antigen with equal quantity of antisera at 1:10 dilution in all possible combinations were mixed thoroughly. The agglutination reaction for all the six isolates of *Ganoderma* in homologous and heterologous combinations were recorded.

RESULTS AND DISCUSSION

Morphological studies

In all 20 basidiocarps of each isolate were examined. One hundred basidiospores from each sporocarp sample were studied and results are presented in Table I. There is some difference between the basidiocarps between the two isolates; however, the basidiospores are more or less alike in colour and size.

Cross inoculation studies

Pin headed brown specks started appearing just above the ground level in four out of ten healthy arecanut palms inoculated with coconut isolate after 20-25 months of inoculation. The lesions started enlarging and coalesced to form brownish gummosis patch in another 30-40 days time. These affected arecanut palms started wilting and dried up in about 30 months after the death of the palm. The fruiting body formed resembled that of the coconut isolate used for inoculation. Hyphae were thick, brownish and did not show much of clamp connections. The description resembles the species *G. applanatum* described by Bakshi (1971). Two coconut palms out of ten inoculated with the arecanut isolate of *Ganoderma* on the other hand, showed development of gummosis patches at the basal portion after 30-35 months of inoculation. These patches started enlarging and turned dark brown later. After 39-40 months of infection sporophores were formed. The sporophores were shiny with laccate crust, reddish brown, perennial, stipitate resembled the sporophores of *G. lucidum* described by Butler (1906), Venkatarayan (1936), and Bakshi (1971).

From the comparison made with the authentic descriptions that both *G. lucidum* and *G. applanatum* could cause infection in coconut an arecanut and are cross inoculable. *G. applanatum*

Table I : Morphological characters of basidiocarps/basidiospores of *Ganoderma* isolate affecting coconut and arecanut

Host from which isolated	Colour	Size	Stalked or sessile	Nature of bracket/spore
Basidiocarps*				
Coconut	Dull brown to dark brown	Large: 12-24 x 8-10 x 4-5	Sessile	Hard, corky, surface uneven, hymenial surface white
Arecanut	Shiny, tan to reddish brown, smooth	Small : 4-12 x 10-12 x 1-4	Stalked Central or lateral	Hard, hymenial surface light brown on drying
Basidiospores**				
Coconut	Yellowish brown	8.8-13.2 x 6.6 - 8.8	-	Broadly ellipsoidal, thick walled, outer wall smooth
Arecanut	Light brown to yellowish brown	8.3 - 10.0 x 5.4 - 6.7	-	Thick walled

* Mean of 20 brackets size in cms
** Mean of 100 spores size in μ

Table II. Agglutination reaction of *Ganoderma* isolates

Antigen	Antisera					
	Areca		Acacia		Coconut	
	Hirahalli	Vittal	Dehradun	Kadar	Hirehalli	Arsikere
	1	2	6	3	4	5
Arecanut	+++	++	+	+++	+++	++
Coconut	+++	++	+	+++	++	+++

+ = Supernatent turbid, ++ = supernatent slightly turbid, +++ = supernatent clear

Table III Gel diffusion reaction showing formation of strong/weak bands between homologous and heterologous antigens of *Ganoderma* isolates

Antigen	Antisera											
	Areca				Acacia		Coconut					
	Agd (H)		Agd (V)		Deh-dun		Cgd (K)		Cgd (H)		Cgd (A)	
	S	W	S	W	S	W	S	W	S	W	S	W
Agd (H)	1	2	1	2	1	1	2	1	2	1	2	1
Agd(V)	1	2	1	2	1	1	2	1	2	1	2	1
Cgd (H)	1	1	1	1	1	1	1	1	3	1	1	1
Cgd (K)	3	2	3	2	1	1	1	3	3	3	1	3
Cgd (A)	2	1	2	1	1	1	1	1	1	1	2	3
Dehradun	1	1	-	1	1	1	1	—	1	1	1	—

S = Strong band; W = Weak band

is a first report from Karnataka on these palm species.

Serological studies

Agglutination test

Appearance of clumps constituted positive tests. Observations were recorded after one hour without disturbing the sediments (Table II). The reaction was recorded as turbid (+), slightly turbid (++) or clear (+++).

Agar gel diffusion tests

Specific antibodies seen as preciptin lines could be detected when the antisera reacted with the antigen. All the six isolates produced precipitin lines against each other. The coconut isolate of Arsikere [Cgd (A) and Kadur Cgd (K)] reacted closely with arecanut and coconut isolates of Hirehalli Agd (H) and Cgd (H) (Table III). The arecanut isolate of Hirehalli Agd (H) reacted with the arecanut isolate of Vittal Agd (V), and with both the coconut isolates Cgd (K) and Cgd (A). The acacia isolate did not react strongly with any of the isolates tested.

The gel diffusion test clearly indicated a fairly high degree of affinity or resemblance between coconut and aracanut isolates. The acacia isolate differed in its identity both with arecanut and coconut isolates. This indicates faint or no antigenic relationship between and with other isolates tested.

It is evident from the above studies that *Ganoderma* species affecting both arecanut and coconut palms in maidan areas of Karnataka are interrelated or related pathotypes.

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DISCUSSION

B. CHANDRAMOULI : How long will it take for the production of initial symptoms on coconut by areca isolate?

S.N. SAMPATHKUMAR : The time lag between disease initiation and symptom expression in coconut palms ranges from 35-38 months.

Y.R. SARMA : It would have been more appropriate to spell out species involved both in coconut and arecanut. If biology is involved it should be with respective species and not general.

S.N. SAMPATH KUMAR : Since the species involves are different, and obtained from two different host plants, in an earlier study, the same have been indicated as isolate. After comparison we have been able to identify it as *G. applanatum*, a new record from Karnataka.