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RESEARCH PAPER

Studies on Colony Morphology and Sporulation of *Ganoderma* Isolates, Causing Basal Stem Rot Disease of Oil Palm

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ABSTRACT

Basal stem rot is an important disease of oil palm caused by different species of *Ganoderma*. A survey has been made in the oil palm gardens of different states and collected suspected BSR affected tissues (root bits / stem tissues / basidiomata). During the study, 11,184 palms were surveyed, (7574 from Andhra Pradesh, 144 from West Bengal and 3466 from Kerala) and 46 palms were suspected to be affected by BSR. All the suspected samples were cultured and 41 isolates could be purified, which were subjected to PCR analysis by using two pairs of *Ganoderma* specific primers namely *Gan*1& *Gan* 2 and ITS3& *Gan* ET. Total 21 isolates (15 from Andhra Pradesh, 3 from West Bengal and 3 from Kerala) were found to be *Ganoderma* positive through PCR analysis. The positive isolates were further grown on Potato Dextrose Agar medium for studying the cultural variability. Growth rate, colony morphology and colony colour were recorded at one-week interval up to third week. Wide variability was observed in the colony morphological characters of the different isolates even among the isolates collected from a specific location. Eight positive isolates from Rajahmundry formed three groups where as three isolates from Palode formed two groups. All the 21 isolates formed five groups based on the colony morphology. Major group containing 14 isolates showed white cottony growth with no colour change at the bottom (under side). All three isolates from Mohitngar (West Bengal) showed same colony morphology. Two of the isolates showed unique colony morphology, one with white thin colony and concentric marks, which later turned to grey and another isolate with white cottony growth initially and later turned to light yellowish having radial corrugations visible on both sides. Out of twenty-one cultures seven of them were only found spore forming. Ellipsoid shape spores were observed in case of six isolates, where as one isolate from Rajahmundry exhibited ovoid shape spore.

INTRODUCTION

Basal stem rot (BSR) is one of the most important diseases of oil palm caused by species of *Ganoderma.* It is the most serious disease of oil palm in Malaysia and Indonesia (Ariffin *et al.*,2000). In India, though the incidences is at present limited in oil palm, but it can be alarming as this disease spreads from other palms. The disease had been reported most often in coastal marine clay, particularly in areas planted with oil palm following coconut (Navaratnam, 1965). Cross infectivity of this disease from coconut is already been observed and reported in India (Hymavathi *et al*., 2002; Mandal *et al*., 2003). Turner (1981) listed 15 species of *Ganoderma* that have been reported from different parts of the world as likely pathogen associated with BSR disease, and he considered single species unlikely to be sole cause of the disease in any particular area. A number of identification systems using cultural, morphological and physiological characters have been devised for mycellial states of the wood-inhabiting *Aphyllophorales* (Miller *et al*., 2000). The majority of the taxonomic studies on species of *Ganoderma* originating from South East Asia have been largely reliant on the system developed by Steyaert (1967, 1972) for defining species. Latiffah and Ho (2005) reported that the morphological characteristics of *Ganoderma* basidiomata from infected oil palms from

three inland estates showed some variations, though all of them fell within the description of *G. boninense.*

Detection of this disease is currently carried out based on the external symptom. Plant infection can only be confirmed when basidiomata of *Ganoderma* appear on the stem base or on infected roots close to the palm (Fig.1), otherwise their disease status is uncertain (Ariffin *et al.,*2000). To handle recent times ELISA is used for prescreening a large number of

Fig. 1 : Basal stem rot affected oil palm with visible *Ganoderma* basidiomata at basal portion of the oil palm

samples, and in case of positive reaction, PCR test can verify the result (Utomo and Niepold, 2000).

So far in India the up to date status of *Ganoderma* from oil palm plantation is not available. In the present study, an exhaustive survey was made in different states and collected the *Ganoderma* affected samples (tissue) from oil palm plantations. The isolates were confirmed by the PCR techniques, and characterized them on the basis of their colony morphology and sporulations.

MATERIALS AND METHODS

Survey

Survey was conducted in the different parts of the country where oil palm is grown. This includes the states of Andhra Pradesh, Karnataka, Tamil Nadu, Kerala, West Bengal, Gujarat and Orissa. Efforts were made to locate individual palms affected with BSR. Samples were collected either from the affected stem / root tissues and the Brackets (basidiomata) from the specific oil palms, and sometimes from the other palms (date, palmyra) and tree (cashew) located in the oil palm gardens. Suspected samples were also collected from the adjacent palms surrounding the affected palm/ trees.

Isolation of *Ganoderma*.

The affected tissues were surface sterilized with 0.1% mercuric chloride for 1 min. washed with sterile distilled water three times, plated on Potato dextrose Agar (PDA) and incubated at 28^{O}C. The *Ganoderma* colonies were subcultured on PDA slants for further studies for obtaining the pure isolates.

Confirmation of isolates by using PCR analysis

All the putative *Ganoderma* isolates purified from the infected oil palm tissues were subjected to DNA extraction and purification by following the method of Mandal *et al*. (2003). Initially *Ganoderma* specific primers *Gan*1 and *Gan*2 (Utomo and Niepold, 2000) were used for the PCR amplification, and the positive

Table 1 : Primers used for diagnosis of *Ganoderma* sp. causing basal stem rot disease of oil palm.

*Gan*1 (Left primer)	5'-TTGACTGGGTTGTAGCTG-3'
*Gan*1 (Right primer)	5'-GCGTTACATCGCAATACA-3'
ITS3 (Left primer)	5'-GCATCGATGAAGAACGCAGC-3'
*Gan*ET (Right primer)	5'-GAGTTGTCCCAATAAC-3'

isolates were reconfirmed with another pair of *Ganoderma* specific primers (Table 1) namely ITS3 and *Gan* ET (Bridge *et al*., 2000).

For both the set of primers, PCR mix was consisting of 20ng of *Ganoderma* genomic DNA, 0.5μM dNTPs (each), 1.5mM $MgCl_2$ (along with 10X buffer), 15 ng of each primers and 1.0 U of enzyme in 25 μl reaction volume. PCR Condition was set as 94^{O}C denaturing temperature, 52^{O}C annealing temperature and 72^{O}C extension temperature for 40 cycles with initial denaturation for 5 minutes and final extension for 10 minutes.

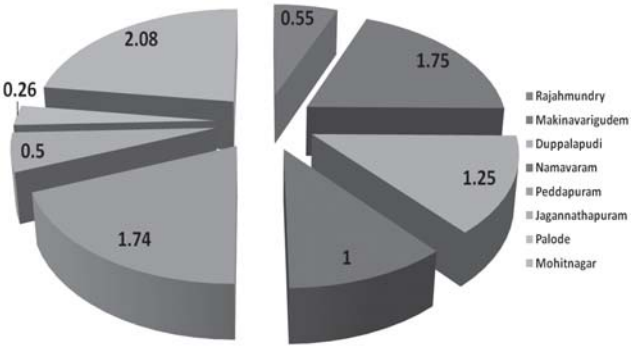
Colony morphology and sporulation studies

All the pure isolates were inoculated at a time in the PDA plates with replications. Growth rate, colony morphology and colony colour were recorded every one-week interval up to third week. Mycelia from three weeks grown culture were treated with 1% 'lactophenol cotton blue' dye and examined under light microscope at 40 X magnification for observing the sporulation pattern.

RESULTS AND DISCUSSION

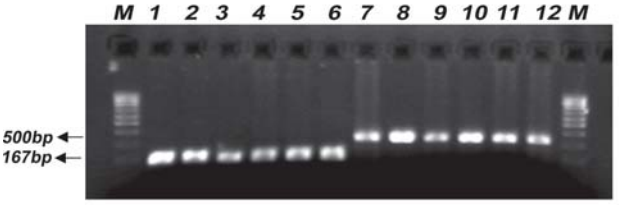
An exhausitive survey of different oil palm growing states had been made during the study. The details of the survey are present in Table 2. As the oil palm is newly introduced crop, the incidence of it is not very high at present, but what is alarming is the cross infectivity from the other crops mainly from other palms. Result showed that total 41 suspected isolates were collected from the oil palm gardens, either from the affected palms/ trees or from the suspected palms. The percentage incidence was not very high at the time of the study (Fig. 2).

Fig. 2 : Percent disease incidence at different locations



For confirmation of the isolates, PCR was carried out with two sets of primers. It was observed that 21 out of 41 isolates were confirmed *Ganoderma* (Fig. 3).

Fig. 3: PCR amplification of Ganoderma isolates with *Gan*1-*Gan*2 and ITS3-*Gan*ET primers. M: 100 bp ladder; Lane 1—6: Ganoderma isolates amplified with *Gan*1-*Gan*2 primers; Lane 7-12: Same *Ganoderma* isolates amplified with ITS3-*Gan*ET primers.



The other isolates, which were purified from the suspected plant tissue, might not be affected by *Ganoderma* but the isolates were some other fungi.

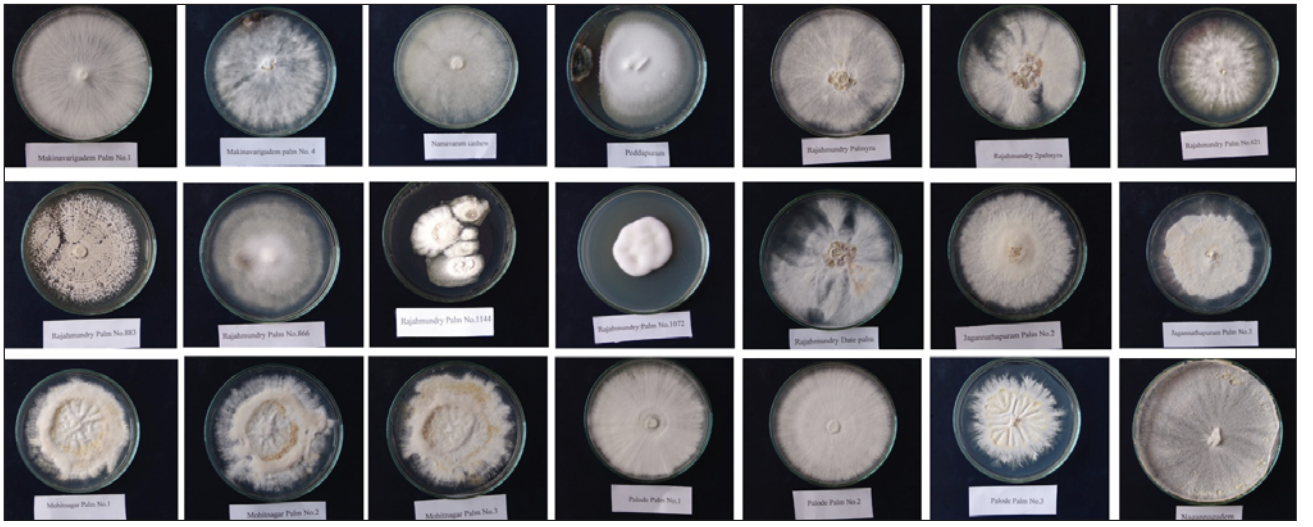
Table 2 : Details of survey made and suspected *Ganoderma* isolates collected

Location/ Planting material	Age	No. of palms	No. of inf. palms	% Disease incd.	Isolates collected	Isolates purified
Andhra Pradesh						
Rajahmundry - Dura	15	2000	11	0.55	13	7
Makinavarigudem–Costa Rica	14	285	5	1.75	5	2
Lova gavaravaram–Costa Rica	16	12500	Nil	Nil	Nil	Nil
Pedavegi – Costa Rica	10	545	Nil	Nil	Nil	Nil
Pedavegi - Pisifera	9	350	Nil	Nil	Nil	Nil
Pedavegi –GXE. Oleifera	7	576	Nil	Nil	Nil	Nil
Pedavegi - Dura	7	780	Nil	Nil	Nil	Nil
Pedavegi - Dura	3 to 5	1110	Nil	Nil	Nil	Nil
Pedavegi –DXG&DXN	2	529	Nil	Nil	Nil	Nil
Duppalapudi – Costa Rica	11	400	5	1.25	5	Nil
Namavaram – PNG	10	400	4	1.00	5	1
Namavaram – PNG	10	400	0	Nil	Nil	Nil
Peddapuram – Costa Rica	10	230	4	1.74	7	1
Jagannathapuram-Costa Rica	14	800	4	0.50	4	2
Nagannagudem Costa Rica	14	260	Nil	Nil	7	1
Kerala						
Palode - D X P	20 to 24	1960	0	Nil	Nil	Nil
Palode - D X P	12 to 24	649	5	0.77	4	1
Palode- T X T	14 to 22	857	4	0.47	3	Nil
West Bengal						
Mohitnagar	17	144	3	2.08	3	2

Table 3 : Grouping based on Colour and Growth habit of different *Ganoderma* isolates

Group	Name of the Isolate	Colour & Growth habit
1	*Gan* 0-1, 2, 3, 4, 5, 6, 7, 9,12, 13, 14, 18,19 and 21	White thin colony, rhizomorph like growth underside also
2	*Gan* 0-15, 16 and 17	Light pink thin cottony growth, underside light brown behind the central disk
3	*Gan* 0-10 and 11	Cottony puffy growth, under side brown corrugations
4	*Gan* 0-8	Grey thin colony with concentric marks, under side also same
5	*Gan* 0-20	Light yellowish, radial corrugations visible on both sides

Fig. 4 : Colonies of *Ganoderma* isolates three weeks after inoculation in the PDA media



When the confirmed isolates were studied for their colony morphology based on colour and growth habit. (Fig.3), five different groups were observed (Table 3).

Positives isolates were from eight locations from three different states (Table 4). However, as per the colony morphology, their grouping did not follow any location specificity (Table 3). Isolates from Rajahmundry were under three different groups and isolates from Palode were under two different groups. Colony morphology of the 21 isolates three weeks after iroculation, are shown in Fig. 4.

Regarding different isolates collected from the same field, vegetative compatibility study made by Miller (1995) and Ariffin *et al.*(1996), indicated that basidiomata collected from the same field or from within the same area of oil palm field, might not have originated from same source of inoculum, implying that root spread of mycelia growth might not the sole method of spread of BSR. Currently the role of *Ganoderma* basidiospores in disease initiation and spread of infection is unclear. Although a huge number of basidiospores of *Ganoderma* are released from basiodiomata in the oil palm field (Ho and Nawawi, 1986), the majority of the oil palm remain unaffected, indicating the basidiospore either may not be able to initiate BSR infection or require very specific condition to establish infection.

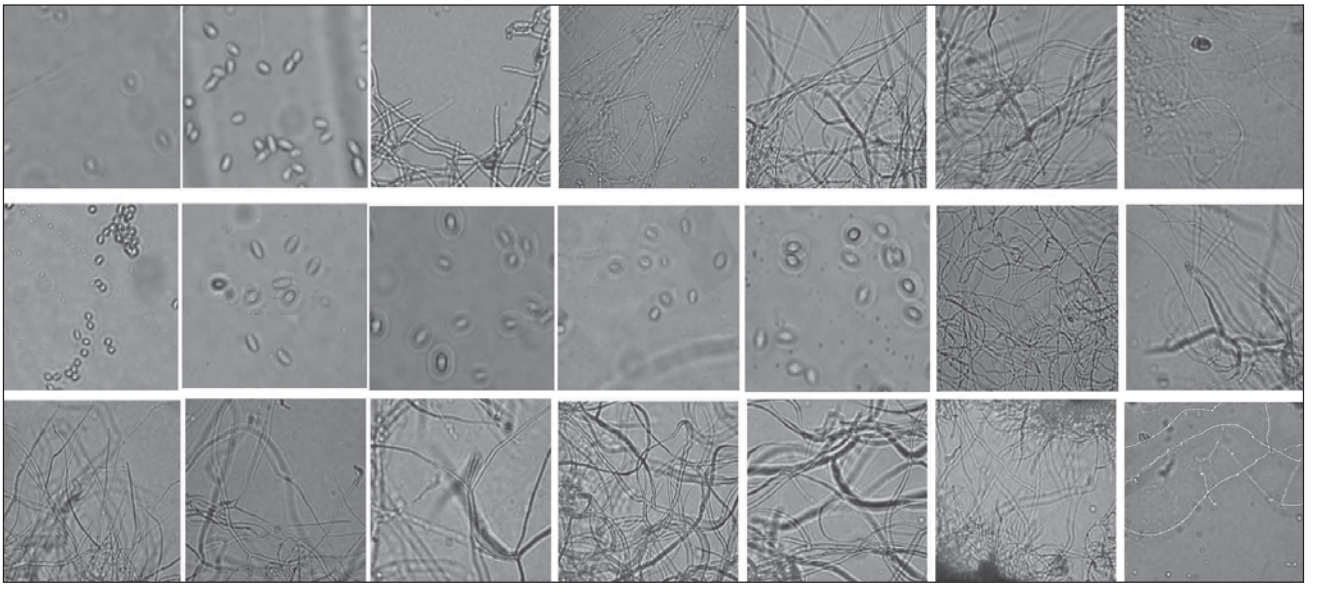
When the sporulation was observed, out of 21 isolates, only 7 isolates formed spores. Ellipsoid shape spores were observed in case of six isolates, where as one isolate from Rajahmundry exhibited ovoid shape spore (Fig 5).

This study reveals that there is variability among the *Ganoderma* isolates causing BSR disease of oil palm in India. However, from the morphological observations the identification of species is not possible. Mycelia identification systems were only of use for identification to the genus level, with parameters insufficiently clear to enable differentiation between species. Miller *et al.* (1995) observed similar variation

Table 4 : List of *Ganoderma* isolates and their location of collection

Isolate ID	Place
Gan0-01, Gan0-02	Makinavarigudem
Gan0-03	Namavaram
Gan0-04	Peddapuram
Gan0-05, Gan0-06 Gan0-07 Gan0-08 Gan0-09 Gan0-10 Gan0-11 Gan0-12 Gan0-13 Gan0-14	Rajahmundry Jagannathapuram
Gan0-15 Gan0-16 Gan0-17	Mohitnagar
Gan0-18 Gan0-19 Gan0-20	Palode
Gan0-21	Nagannagudem

Fig. 5 : Mycelial growth and sporulation of *Ganoderma* isolates



levels intraspecifically and interspecifically indicating inapplicability for species definitions, and in differentiation of populations in the context of functional characteristics, such as host specificity on tropical perennial crops. Diagnosis of *Ganoderma* infection in tropical perennial hosts such as oil palm thus remains largely reliant on presence of basiodiomata which frequently appear only once the disease is firmly established (Miller *et al*., 2000). However, more recent PCR-based approaches such as RAPD, AFLP or microsatellite may be appropriate for clarification of BSR disease establishment and pathogen spread in oil palm (Miller *et al*., 2000, Mandal *et al*., 2003). These approaches are being used to discriminate individual isolates with a reduced cost and handling time at this centre.

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