International Journal of Oil Palm, **8 (1&2)** : 23-26, June and December, 2011

RESEARCH PAPER

Development of Molecular Diagnostic Kit for Early Detection of Basal Stem Rot in Oil Palm (*Elaeis guineensis* Jacq.)

K. Praveena Deepthi [1*], R. Chowdappa [2], P.K. Mandal[3] and Nirmal Kumar[2]

[1]Directorate of Oil Palm Research, Pedavegi, Andhra Pradesh, India.
[2] Indian Institute of Horticultural Research, Hassaragatta, Bangalore, Karnataka, India.
[3]Indian Agricultural Research Institute, New Delhi, India.
*praveenadeepthi@gmail.com

ABSTRACT

A study was carried out to develop a molecular diagnostic kit for early detection of basal stem rot disease before manifestation of symptoms. A survey on incidence of basal stem rot in India was carried out and the incidence varied from 2.9% to 52.85%. During the survey, 35 isolates were collected and used for the study. The *Ganoderma* specific primers *gan-1* (3'-TTGACTGGGTTGTAGCTG-5') and *gan-2* (5'-GCGTTACATCGCAATACA-3') that are already validated against *Ganoderma* are proved specific for oil palm *Ganoderma* also. The set of primers proved non specific to other oil palm fungi. A methodology for PCR amplification of oil palm *Ganoderma* DNA was standardised with a recipe of primers each of 1 µl, taq polymerage 0.25 µl, taq buffer 5 µl, dntps 1 µl, template 1 µl. The correct tissue to be sampled for diagnosing the basal stem rot is identified. A methodology for DNA isolation from the affected oil palm tissue is developed and the disease can be diagnosed using the above primers.

Key words : Oil palm, basal stem rot, diagnosis, PCR

INTRODUCTION

Oil palm (*Elaeis guineensis*) is one of most important plantation crops being subjected to a substantial loss of yearly harvests due to *Ganoderma* species, the casual agent of basal stem rot (BSR) disease. BSR was first reported in 1930 in Malaysia and was identified as *Ganoderma lucidum* (Thompson, 1930). One of the limiting factors in controlling the disease is the lack of reliable diagnostic methods to detect early symptoms of BSR disease. Only two methods have been developed so far for early diagnosis of BSR. One involves a colorimetric method using ethylenediaminetetraacetic acid (EDTA) to detect *G. lucidum* in coconut, the casual agent of Thanjavur wilt disease (Natarajan *et.al*., 1986). The second is a drilling technique (Ariffin *et.al.,* 1993). These conventional methods are time consuming and the accuracy is not very high. Therefore, the availability of a rapid, inexpensive and accurate diagnostic technique, which is specific and readily adapted to large scale testing for demonstrating *Ganoderma* in oil palm at an early stage of infection, would benefit decision-making for appropriate control. The development of molecular biology methods that allow the detection and analysis of DNA sequences has resulted in a considerable increase in the accuracy and speed of fungal identification.

More recently, internal transcribed spacer (ITS) regions of ribosomal DNA (rDNA) have been targeted as attractive tools for early detection, due to their high sequence variation between species and their general conservation within any one species (Tisserat *et.al.,* 1994; Lovic *et.al.,*1995). Therefore, this present study was carried out to detect *Ganoderma* using the ITS primers. Apart from this, best tissue suitable for detection and sampling procedure were also standardised for effective detection. The results of the study will be useful in rapid and early detection of the disease.

MATERIALS AND METHODS

Survey of Basal Stem Rot affected fields

Basal stem rot affected oil palm fields were surveyed for estimating the incidence and intensity of the disease. Simultaneously, the samples required for the study were collected. During the survey, 35 samples of *Ganoderma* from BSR affected palms were collected.

Standardisation of diagnostic protocol using already validated and reported primers

The fungal isolates were isolated from the fruiting bodies collected during the survey through the tissue segment method (Aneja, 2003) and further maintained on Potato Dextrose Agar. The DNA of all the collected samples were isolated (Raeder and Broda, 1985). The protocol of PCR amplification for basal stem rot DNA with the already available *Ganoderma* specific primers *gan-1* (3'-TTGACTGGGTTGTAGCTG-5') and *gan-2* (5'-GCGTTACATCGCAATACA-3') was standardised by using a series of primers (0.5 µl, 1.0 µl, 1.5 µl, 2.0 µl), dNTPs (0.5 µl, 1.0 µl, 1.5 µl, 2.0 µl), taq polymerase (0.1 µl, 0.25 µl, 0.5 µl, 1. 0µl) and template concentrations (0.5 µl, 1.0 µl, 1.5 µl, 2.0 µl) in a *Genetix palm cycler* machine. The PCR products were analysed by electrophoresis on a 2% agarose gel followed by visualization under UV light and documented using a gel documentation system (*Genei Uvitec, Cambridge)*.

Testing the reported *Ganoderma* specific primers against closely related fungal species

After standardising the protocol, the primers were cross checked with other fungal pathogens of oil palm by following the standardized protocol for PCR amplification. The DNA of related fungi and pathogens were isolated and amplified following the standardised PCR recipe and programme presented in the previous objective.

Identification of correct tissue to be sampled for detection of *Ganoderma* from affected oil palm

The samples of different tissues of affected palms *viz.,* root, basal stem, upper stem and leaf were collected. The population of *Ganoderma* was estimated from each sample by following a 10-fold serial dilution and plating method. The population was recorded in terms of colony forming units (cfu). The DNA of all the samples were isolated and amplified with the standardised protocol. Based on the cfu and amplification in PCR reaction, the best tissue to be sampled for detection of basal stem rot was identified.

RESULTS AND DISCUSSION

Survey of Basal Stem Rot affected fields

A survey on incidence of basal stem rot in Andhra Pradesh was done in Kuchimpudi, Pedavegi, Laxmipuram, Medinaraopalem, Venkataramanna gudem, Tallagokavaram villages in West Godavari distrct; Rajamundry and Kadiyapulanka in East Godavari district; Nujividu, MNpalem in Krishna district; Aswaraopet and Naaramvarigudem in Khammam district; Parvathipuram in Vizianagaram district; J D peta in Vizag district. The incidence varied from 2.9% to 52.85%.

Standardisation of diagnostic protocol using already validated and reported primers

From the above affected fields, 35 *Ganoderma* samples were collected and used for the studies. The ganoderma specific primers *gan-1* (3'-TTGACTGGGTTGTAGCTG-5') and *gan-2* (5'-GCGTTACATCGCAATACA-3') that are already validated were proved specific to *Ganoderma* isolated from oil palm also by producing a band with a molecular weight of 167 bp. All the 35 samples were amplified in PCR reaction with a recipe of primers each of 1 µl, taq polymerage 0.25 µl, taq buffer 5 µl, dNTPs 1 µl, template 1 µl. The PCR machine was programmed as, 4 min preheating at 94^0C followed by 34 cycles consisting of denaturation at 94^0C for 1 min, annealing at 55^0C for 1 min and extention at 72^0C for 1.5 min with a final 5 min extention at 72^0C.

Testing the reported *Ganoderma* specific primers against closely related fungal species

The primers were cross checked for their cross reaction with other fungi associated with oil palm like *Schizophyllum sp, Marasmius palmivora, Glomerella cingulata, Colletotrichum, Pestalotia palmarum* and other saprophytes like *Fuasrium, Botryodiplodia theobromae* etc. The DNA of these fungi were isolated and kept for PCR amplification by following the above standardized procedure. But none of the fungal DNA were amplified (Fig.1). The standardized composition for PCR reaction is specific to *Ganoderma* sp.

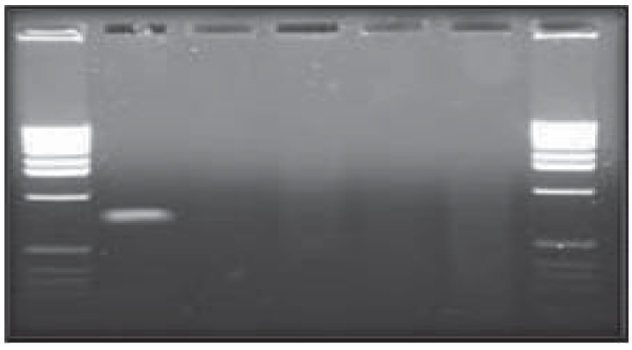

Fig. 1 : Gan-1 and gan-2 primers are specific to *Ganoderma lucidum*, not to other fungi of oil palm. Lane 1: *Ganoderma lucidum*; Lane 2: *Phellinus noxius*; Lane 3: *Marasmius palmivorus*; Lane 4: *Glomerella cingulata*; Lane 5: *Pestalotia palmarum*

Identification of correct tissue to be sampled for detection of *Ganoderma* from affected oil palm

The correct tissue to be sampled for diagnosing the basal stem rot is identified. Samples from 25 BSR affected palms were taken from four locations of the palm like root, basal stem, upper stem and leaf. Among the tissues, basal stem tissue recorded highest mean colony forming units of *Ganoderma lucidum* (2.52 x 10^6 /g) with 96% occurance of amplification in PCR reaction (Table 1, Fig. 2) with *gan-1* and *gan-2* primers. Minimum *Ganoderma* population was observed in leaf tissue (0.32 x 10^6 /g) with 0 % occurance of amplification in PCR reaction.

A methodology for DNA isolation from the affected oil palm tissue was developed. The DNA can

Table 1: Population of *Ganoderma lucidum* in different tissues of oil palm

Palm no	Leaf *cfu /g	Presence of band	Root cfu /g	Presence of band	Basal stem cfu /g	Presence of band	Upper stem cfu /g	Presence of band
1	-	No	1 x 10^6	No	3 x 10^6	Yes	-	No
2	-	No	2 x 10^6	Yes	3 x 10^6	Yes	-	No
3	-	No	2 x 10^6	Yes	2 x 10^6	Yes	-	No
4	-	No	1 x 10^6	No	3 x 10^6	Yes	1 x 10^6	No
5	1 x 10^6	No	1 x 10^6	Yes	2 x 10^6	Yes	1 x 10^6	No
6	-	No	1 x 10^6	Yes	2 x 10^6	Yes	1 x 10^6	No
7	-	No	2 x 10^6	Yes	1 x 10^6	Yes	-	No
8	-	No	2 x 10^6	No	1 x 10^6	No	-	No
9	1 x 10^6	No	3 x 10^6	Yes	3 x 10^6	Yes	2 x 10^6	No
10	-	No	1 x 10^6	Yes	2 x 10^6	Yes	1 x 10^6	No
11	-	No	2 x 10^6	Yes	3 x 10^6	Yes	-	No
12	-	No	2 x 10^6	Yes	3 x 10^6	Yes	-	No
13	-	No	2 x 10^6	Yes	3 x 10^6	Yes	-	No
14	-	No	3 x 10^6	No	3 x 10^6	Yes	-	No
15	1 x 10^6	No	3 x 10^6	Yes	3 x 10^6	Yes	-	No
16	1 x 10^6	No	2 x 10^6	Yes	3 x 10^6	Yes	1 x 10^6	No
17	2 x 10^6	No	3 x 10^6	Yes	2 x 10^6	Yes	2 x 10^6	No
18	-	No	2 x 10^6	Yes	2 x 10^6	Yes	-	No
19	-	No	2 x 10^6	Yes	2 x 10^6	Yes	-	No
20	-	No	2 x 10^6	No	3 x 10^6	Yes	-	No
21	1 x 10^6	No	3 x 10^6	Yes	4 x 10^6	Yes	-	No
22	1 x 10^6	No	2 x 10^6	Yes	3 x 10^6	Yes	2 x 10^6	No
23	-	No	1 x 10^6	No	3 x 10^6	Yes	1 x 10^6	No
24	-	No	1 x 10^6	No	2 x 10^6	Yes	1 x 10^6	No
25	-	No	2 x 10^6	Yes	2 x 10^6	Yes	-	No
Mean	**0.32 x 10^6**		**1.92 x 10^6**		**2.52 x 10^6**		**0.52 x 10^6**	
Percent occurrence of band	**0**		**72**		**96**		**0**	
Mean		**CD(0.05) 0.45**			**SeM ± 0.44**			
Percent occurrence		**CD(0.05) 15.83**			**SeM ± 1.38**			

***Colony Forming Units/gram**

be isolated from basal stem tissue by Raedar and Broda (1985) method and the disease can be diagnosed using the *gan-1* and *gan-2 Ganoderma* specific primers.

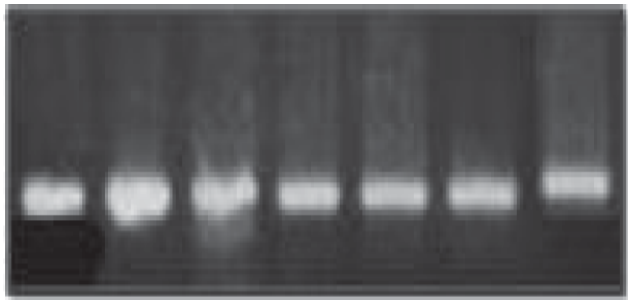

Fig. 2: Detection of *Ganoderma lucidum* from basal stem tissue

Development of procedures for a species specific detection of *Ganoderma* sp. in oil palm is important for early detection of basal stem rot. Because it is important for crop rotations with coconut, cashew nut etc., as their stumps are usually left in the field from which the basal stem rot is being spread. However it is very difficult to detect whether the *Ganoderma* sp colonizing these stumps represent the same species which can also infect healthy oil palms. So the molecular method is tried for diagnosis of *Ganoderma* with PCR. Application of the two *gan-1* and *gan-2* primers generated from the ITS1 sequence proved to be useful for detection of pathogenic *Ganoderma* isolates. The interspecific variation of the ITS sequences was high enough to differentiate between other fungi of oil palm (Utomo and Niepold, 2000). The ITS1 region of *Ganoderma* is flanked by highly conserved sequences (Moncalvo *et al.,* 1995).

Ganoderma specific primers *gan-1* and *gan-2* are specific to oil palm *Ganoderma* also. The set of primers are not cross reacting with the DNA of any other fungus associated with oil palm. Basal stem tissue is proved to be the best for detecting the fungus. Basal stem rot can be diagnosed with the *gan-1* and *gan-2* primers by isolating the DNA through Raeder and Broda method and amplifying in PCR through the standardized procedure.

ACKNOWLEDGEMENTS

The first author is thankful to Dr. S. Arulraj, Director, DOPR, Pedavegi for providing necessary facilities and encouragement to carry out the study.

REFERENCES

Aneja, K. F. 2003. Experiments in Microbiology, Plant Pathology and Biotechnology (4th edition). New Age International Publishers. pp. 66-73.

Ariffin, A., Seman, I.A. and Azahari, M. 1993. Spread of *Ganoderma boniense* and vegetative compatibility studies of a single field palm isolates. In: 1996 PORIM *International Palm Oil Congress, 23-28 September 1996*, Kuala Luampur, Malaysia.

Lovic, B. R., Martyn, R. D. and Miller, M. E. 1995. Sequence analysis of the ITS regions of r DNA in *Monosporascus spp.* to evaluate its potential for PCR-mediated detection. *Phytopath.* **85**: 655-661.

Moncalvo, J. M., Wang, H. H. and Hseu, R. S. 1995. Phylogenetic relationships in *Ganoderma* inferred from the internal transcribed spacers and 25S ribosoma DNA sequences. *Mycologia*. **87**: 223-238.

Natarajan, S., Bhaskaran, R. and Shanmugam, N. 1986. Preliminary studies to develop techniques for early detection of Thanjavur wilt in coconut. *Ind. Coco. J.* **17**: 3-6.

Raeder, U. and Broda, P. 1985. Rapid preparation of DNA from filamentous fungi. *Letters of App. Microb.* **1**: 17-20.

Thompson, A. 1930. Stem rot of the oil palm in Malaya. *Bull. of Dept. of Agril Sci. Series*, 6.

Tisserat, N.A., Hulbert, S. H. and Sauer, K. M. 1994. Seletive amplification of rDNA internal transcribed spacer regions to detect *Ophiosphaerella korrae* and *oherpotricha. Phytopath.* **84**: 478-482.

Utomo, C. and Niepold, F. 2000. Development of diagnostic methods for detecting *Ganoderma* infected oil palms. *J. of Phytopath*. **148**: 507-514.