Short scientific report

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Early diagnosis of *Ganoderma* infection in indicator plants through ELISA and visual observation

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Basal Stem Rot (BSR) disease in coconut incited by the fungus *Ganoderma lucidum* (Leys) Karst (Bhaskaran and Ramanathan, 1983) is one of the main limiting factors in coconut cultivation. It is a severe disease in India and in some cases the incidence as high as 31% was recorded (Anonymous, 1987). The disease is responsible for significant reduction in yield of nuts every year in India and in some Asian countries (Bhaskaran *et al.*, 1984). Management practices are effective only when the disease is diagnosed at the early stages. Trees can be saved from the disease if reliable diagnostic methods are available. A few methods have been reported to be useful for early diagnosis of the disease (Vijayaraghavan *et al.* 1987). A colorimetric method using ethylene diamine tetra acetic acid (EDTA) was reported by Natarajan *et al. (*1986) in which optical density of the sap from infected stem tissues was found to increase with increase in disease intensity. Use of indicator plants for the early detection of basal stem rot in coconut was also reported (Srinivasulu *et al.*, 2006). In the present study attempts were made to test the use of DAC-ELISA in early detection of BSR disease and also by using indicator plants.

An experiment was laid out for early diagnosis of *Ganoderma* in indicator plants. The basal stem rot infected (in BSR sick plot) and healthy coconut palms were selected at Coconut Research Station, Veppankulam to conduct the experiment. The seeds / cuttings of indicator plants such as *Cajanus cajan* (seeds), *Sesbania rostrata* (seeds),*Leucana leucocephala* (seeds) and *Glyricidia* (cuttings) were sown / planted in the basins of basal stem rot infected and healthy (apparently healthy) palms separately.

The same experiment was also conducted under glasshouse conditions with only *Cajanus cajan* and *Glyricidia* sp. using sterilized soil with artificial inoculation of *Ganoderma* isolates (TKT 1 and CRS 1). The inoculum of the isolates were multiplied on sorghum grains and inoculated at the time of sowing / planting @ 300 g / pot. Root samples from both field and pot culture experiments were collected at monthly intervals. The early diagnosis technique *viz.*, Direct Antigen Coating - Enzyme Linked Immuno Sorbant Assay (DAC -ELISA) was done for the root samples as per the method described by Viswanathan *et al.* (1998). The antiserum used in the present study was specific to *Ganoderma* and it was obtained from the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore. Observations on the appearance of external symptoms in the indicator plants were also made at regular intervals.

There was an increase in absorbance values of indicator plants inoculated with *Ganoderma* under pot culture conditions and also the plants grown in BSR infected tree basins under field conditions compared to their respective counter parts (Table 1).

Three-fold increase in absorbance value was observed in *Glyricidia* inoculated with *Ganoderma* than the un-inoculated control plants. The methods presently available for the early diagnosis of BSR in coconut are Colorimetric method, EDTA test (Natarajan *et al.*, 1986), Orthophenanthroline reagent test (Anonymous, 1989) and transpiration rate test (Vijayaraghavan *et al.*, 1987). Among these four methods, the EDTA method and the Orthophenanthroline reagent method have certain lacunae. In both these tests, the iron content of the extracts was more in diseased tissues than apparently healthy roots.

However, within the different disease categories, the iron content decreased with increase in disease severity in EDTA test while in Orthophenanthroline test, the iron content of the extract increased with increase in disease severity (Anonymous *et al.*, 1989). But in the present study, the indicator plants, whether grown under pot culture conditions or under field condition, showed

Table 1. Early diagnosis of *Ganoderma* through DAC - ELISA in indicator plants

Sl. No.	Treatments	Absorbance value (A_{405}) at different month after sowing / planting* Mean
Pot culture (Glasshouse) - Inoculated		
1.	Cajanus cajan	2.518
2.	Sesbania rostrata	1.685
3.	Glyricidia sp	1.216
4.	Leucana leucocephala	1.296
Un- inoculated		
1.	Cajanus cajan	1.801
2.	Sesbania rostrata	1.400
3.	Glyricidia sp.	0.362
4.	Leucana leucocephala	2.155
Field condition - BSR infected tree basins		
1.	Cajanus cajan	2.493
2.	Sesbania rostrata	2.976
3.	Glyricidia sp	2.351
4.	Leucana leucocephala	0.760
Healthy tree basins		
1.	Cajanus cajan	0.714
2.	Sesbania rostrata	0.618
3.	Glyricidia sp	0.805
4.	Leucana leucocephala	0.810
Root samples of the palm		
1.	Infected	0.319
2.	Healthy	0.173
	CD (P=0.05)	0.086

*Mean of two replications

increased absorbance values compared to the healthy plants. Hence, from the results of the present study, it is concluded that DAC- ELISA could be employed as an early diagnostic method for the detection of BSR in coconut through indicator plants.

By visual observation, the latent inspection of BSR can be detected through the appearance of external splitting symptom on the stem base of red gram plants at one month after sowing near the diseased palms. In the red gram plants near healthy palms this symptom was not noticed. Further, the sporophore of *Ganoderma* has appeared at the stem base of the red gram grown in the BSR infected tree basin at four months after sowing (Fig.1). Hence, it is further concluded that wherever the laboratory facilities are not available for conducting DAC – ELISA and under farmers' field conditions, red gram plants can be used as an indicator plant for the early detection of BSR in coconut.

Fig. 1. Reaction of red gram (Cajanus cajan) as indicator plant in Ganoderma sick soil

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