Review Article

Review of Genus *Ganoderma* causing Basal Stem Rot (Coconut) and Foot Rot (Arecaanut) with Respect Etiology and Management

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A B S T R A C T

*Ganoderma* species are important wood decaying fungi distributed throughout the world. They are diverse in the tropics affecting plantation crops such as coconut, arecanut and oil palm and they also affect ornamental, forest and avenue trees in tropical and temperate areas causing Basal Stem rot/*Ganoderma* wilt. Basal Stem Rot (BSR), also called *Ganoderma* wilt in coconut and foot rot in arecanut caused by the different species of *Ganoderma* is one of the most devastating diseases affecting production and productivity of palms. This review aims to give a broad overview on occurrence and distribution; etiology; identification of species; virulence analysis; disease development; early detection and latest developments with respect integrated disease management aspects of *Ganoderma* wilt and to highlight interesting findings as well as promising applications.

Keywords
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Introduction

Coconut (*Cocos nucifera* L.) and Arecanut (*Areca catechu* Linn.), belonging to family Arecaceae are important commercial crops of India providing livelihood to a substantial number of farm families. Coconut, the versatile palm popularly known as ‘King of Palms’, ‘Tree of Heaven’, ‘Tree of life’, ‘Tree of Abundance’, as well as ‘God’s gift to mankind’, is grown in more than 93 countries within an area of 12.8 million hectares and production of 10.9 m MT (copra equivalent) in 2001.

The total area and the production in Asian Pacific Coconut Committee (APCC) countries are estimated at 11.4 mha and 9.2 m MT, which is 90 and 84 per cent of world area and production (Rethinam and Taufikkurahman
Coconut palm is often described as ‘Kalpavriksha’ because of the multifarious uses of every part of it in the commercial sector. It is equally important in the therapeutical sector, especially in medicine. The natural habitat of coconut is the coastal belt of tropics where it flourishes in seawashed littoral sand with constant motion of underground current of water in rhizosphere.

In India coconut palms are grown in an area of 1.94 million hectares with a production of 14811.4 million nuts and a productivity of 7608 nuts/ha. annually. Kerala ranks first in terms of area and production followed by Tamil Nadu, Karnataka and Andhra Pradesh, while, Tamil nadu ranks first in the productivity followed by Andhra Pradesh and Kerala. The coconut palm exerts a profound influence on the rural economy of the many states where it is grown extensively and provides sustenance to more than 10 million people in the country.

Areca nut is a tropical plant found all over South-East Asia. The fruit (nut) of this tree is popularly known as the betel nut or supari in India. This is an important commercial crop of the region and also forms part of ritual offerings in Hindu religion. Areca is taken up from the Malayan language which means ‘cluster of nuts’. The current production of arecanut in the world is about 127 thousand tonnes from an area of 925 thousand ha. India ranks first in both area (49 %) and production (50 %) of arecanut. Other major arecanut producing countries are Indonesia (16 % area and 15 % production), China (5 % area and 11 % production) and Bangladesh (20 % area and 8 % production). In India, arecanut is cultivated in an area of 4.53 lakh hectares with an annual production of 6.32 lakh tonnes. In India, arecanut is popular for masticatory purpose and is chewed either with betel leaves or as scented supari. Arecanut cultivation is concentrated in South western and North eastern regions. The major states cultivating this crop are Karnataka (40 %), Kerala (25 %), Assam (20 %), Tamil Nadu, Meghalaya and West Bengal which accounts for more than 70 per cent of the area and production.

Coconut and arecanut palms are normally affected by various biotic and abiotic stresses resulting in drastic reduction in yields. Among the various biotic stress that affect coconut and arecanut production in India, Basal Stem Rot (BSR) or Ganoderma wilt caused by Ganoderma applanatum Pers and G. lucidum Leys. Karst. is a major constraint in coconut and arecanut production, especially in dry tracts of Southern Karnataka. The disease is reported from various places all over the tropical world viz., India, Sri Lanka, West Indies, Seychelles, Guam etc. Though the disease was first recorded by Dr. Butler in the beginning of 20th century and later by Venkatanarayan (1936) from Karnataka, a severe outbreak occurred in 1652 in Thanjavur district of Tamil Nadu, and hence named as Thanjavur wilt. The disease is also reported from Andhra Pradesh (Basal stem rot), Kerala, Maharashtra, Gujrat and Orissa (Bhaskaran, 1994; Wilson et al., 1987). In arecanut it was called as Foot rot (Anabe roga in Kannada) and was reported by Coleman from Karnataka in 1911. This disease is also reported from Kerala, Assam, West Bengal (Sharples, 1928) and Nicobar Islands (Sangal et al., 1961) and also from parts of Tamil Nadu.

The genus Ganoderma was introduced by Finnish mycologist Peter Adolf Karsten in 1881 (Karsten, 1881), with G. lucidum (Curtis:Fr.) P. Karst from England as the type species. The genus belongs to the family Ganodermataceae that resides in the order Polyporales of the Basidiomycetes. The family includes eight genera that are distinguished by their unique double-walled
basidiospores. The genus *Ganoderma* was further subdivided into two subgenera: subgenus *Ganoderma* based on *G. lucidum* for the laccate species and subgenus *Elfvingia* based on *G. applanatum* for the species with a non-laccate fruiting body (Moncalvo and Ryvarden, 1997). Thus, *G. lucidum* and *G. applanatum* are the two important species complexes in the history and nomenclature of the genus. These are two of the most poorly understood species of *Ganoderma* and most frequently with misapplied names (Seo and Kirk, 2000).

*Ganoderma* species are important wood decaying fungi occurring throughout the world. They are diverse in the tropics affecting plantation crops such as coconut, arecanut and oil palm by causing Basal Stem rot (Singh, 1991; Ariffin *et al*., 2000; Flood *et al*., 2000; Pilotti *et al*., 2003; Pilotti, 2005) and they also affect ornamental and forest trees in tropical and temperate areas causing disease and wood rots of timber (Lee, 2000). These fungi cause white-rot of hardwoods through delignification (Adaskaveg *et al*., 1991, 1993; Schwarze *et al*., 1995). In the early stages of decay caused by *Ganoderma* species, bleached zones usually appear in the wood, as a result of selective delignification. As the decay progresses, the wood becomes softer and loses tensile strength until a late stage where the wood disintegrates and becomes soft or spongy (Schwarze and Ferner, 2003).

The *Ganoderma* spp. has a wide host range attacking variety of palms and several forest, avenue and fruit trees (Naidu *et al*., 1966., Govindu *et al*., 1983; Bhaskaran, 1994). It usually attacks old or weak palms growing under unfavourable conditions. It is a soil dweller inhabiting dead as well as living plant material in the soil, enters through the wounds and spread of the disease takes place mainly through soil. Its incidence was observed maximum (up to 62.50 %) in coconut gardens raised in sandy and red soils in coastal district of Andhra Pradesh (Srinivasulu *et al*., 2003).

Basal Stem Rot (BSR), also called *Ganoderma* wilt, of coconut and arecanut caused by the different species of *Ganoderma* is one of the most devastating diseases of numerous perennial, coniferous and palmaceous hosts. In forest systems, *Ganoderma* has an ecological role in the breakdown or delignification of woody plants. BSR disease takes several years to develop and for expression of symptoms, presence of the pathogen is often only visible by fruiting bodies when the fungus is well established and more than half of the bole tissue decayed, leaving no chance for the grower to cure the infected palms resulting in drastic reduction in production and productivity of the palms (Kandan *et al*., 2010). The genus *Ganoderma* belongs to the family of Ganodermataceae, which causes white rots of hardwoods in many woody plants by decomposing lignin as well as cellulose and related polysaccharides (Hepting, 1971). *Ganoderma* is an economically important plant pathogen especially in coconut, arecanut as well as in oil palm. A literature review concerning occurrence and distribution, disease development and symptoms, isolation and establishment of pure culture, disease diagnosis, taxonomy, morphological, cultural and molecular characterization besides integrated disease management practices are reviewed here under:

### Occurrence and distribution

The distributions of *Ganoderma* species are worldwide in green ecosystem both to tropical and temperate regions (Pilotti, 2005). The causal pathogen was originally identified as *Ganoderma lucidum* Karst in West Africa (Wakefield, 1920). In Malaysia, the causal pathogen was first recognised as *G. lucidum*
The species *Ganoderma* has a wide host range, where more than 44 species from 34 genera of plants have been identified as potential hosts (Venkatanarayan, 1936). This includes the coconut and oil palms as the main hosts for BSR (Hasan and Turner, 1998). Turner (1981) reported fifteen species of *Ganoderma* from various parts of the world such as North America, Africa, India, Malaysia, Indonesia, Papua New Guinea and Thailand as being associated with BSR, that includes, *G. applanatum*, *G. boninense*, *G. chalceum*, *G. cochlear*, *G. lucidum*, *G. miniaticinctum*, *G. pseudoferreum*, *G. tornatum*, *G. tropicum* and *G. zonatum*. Within these species *G. boninense*, *G. chalceum*, *G. lucidum*, *G. miniaticinctum*, *G. pseudoferreum*, *G. tornatum* in diseased oil palms from different areas of Peninsular Malaysia, *G. boninense* is the most aggressive pathogen to causing the basal stem rot in oil palm (Wong et al., 2012; Turner 1981).

*Ganoderma* is a diverse fungus which occurs in its natural habitat throughout the world. Over 250 species have been described worldwide (Moncalvo and Ryvarden 1997). *G. lucidum* grows near stumps of oak and other broad leaved tree species in summer and autumn in the wild. It may also grow saprophytically or parasitically on logs (Singh et al., 2007). *G. lucidum* grows on a wide variety of trees as a pathogenic fungus during monsoon season. It is distributed in tropical and warm temperate areas of India. Its fruiting bodies have gained wide popularity not only in China, Japan and USA but in all other parts of world (Doshi and Sharma, 1997).

The disease has been reported from various places all over the tropical world viz., India, Sri Lanka, West Indies, Seycheles, Guam, etc. Though the disease was first recorded by Dr. Butler in the beginning of 20th Century, and later by Venkatanarayan (1936) from Karnataka, a severe out break occurred in 1952 in Thanjavur district of Tamil Nadu. Till 1960s, the disease was confined to the coastal areas of Tamil Nadu. In 1978, the disease was noticed in all the districts of Tamil Nadu (Bhaskaran and Ramanathan, 1984). In severely affected gardens in Thanjavur district, the incidence was as high as 31 per cent (Bhaskaran et al., 1984). Apart from Tamil Nadu, the disease is reported from Andhra Pradesh (Srinivasalu et al., 2003), Karnataka (Govindu et al., 1983), and Kerala, Maharastra, Gujrrath and Orissa (Bhaskaran et al., 1994; Wilson et al., 1987). *Ganoderma* sp. has a wide host range attacking variety of palms and several forest, avenue and fruit trees (Naidu et al., 1966; Govindu et al., 1983; Bhaskaran et al., 1994).

The fungus usually attacks old or weak palms growing under unfavorable conditions. The pathogen is a soil dweller inhabiting dead as well as living plant material in the soil, enters through the wounds and disease spread mainly through soil. Basal stem rot disease incidence ranged from 6.06 to 36.15 per cent in Arasikere Taluk of Karnataka (Naik et al., 2000) and maximum incidence (62.50 %) was observed in coconut gardens raised in sandy and red soils in coastal district of Andhra Pradesh (Srinivasulu et al., 2003). The incidence of foot rot of arecanut during the year 2014–15 ranged from 0 to 55 per cent in southern dry tracts of Karnataka. Among the four districts surveyed, Tumkur recorded the maximum disease incidence (19.3%) followed by Chitradurga (18.1%) and Hassan (4.3%) (Palanna et al., 2018).

**Disease development and symptomology**

*Ganoderma* infects palm from seedlings to old plants, the disease progresses slowly and every infected plant eventually dies soon or later. The infection begins from the roots but
the external symptoms appear on young palm as one-sided yellowing or molting of lower fronds, followed by necrosis (Singh, 1991; Kandan et al., 2010). The newly unfolded leaves were shorter and chlorotic and some times the tips necrotize. Infected older plants produced several unopened spear leaves that were usually white in color. By the time the foliar symptoms apparent, the fungus killed half of the plant’s tissues (Arrifin et al., 2000). The roots of the infected palms were very friable and their internal tissues become very dry and powdery. The cortical tissue was brown and disintegrated easily, while the stele becomes black in color (Singh, 1991).

Infected young palms died within 6 – 24 months, whereas mature palms took 2–3 years (Arrifin et al., 2000). A glasshouse trial study found that the disease severity of 8.3% in roots on six-month-old oil palm seedlings however, leaf with no external symptoms (Naher et al., 2012a). BSR is called a silent killer of palms as the basal tissues in cross section showed as brown areas of rotting, wherein leaves appeared alive. Turner (1981) and Ariffin et al., (1989) reported that areas of rotting tissues or darker yellow zones as ‘reaction zones’ that result from defence mechanisms of the palm to form infection.

Ganoderma causes root and stem rot disease resulting in losses of crops such as oil palm, rubber and other trees worldwide (Chee, 1990; Singh 1991; Ariffin et al., 2000; Lee, 2000; Sankaran et al., 2005). The disease could easily be detected when fruiting bodies (basidiocarps) appear on the stem of a tree, which release basidiospores (Pilotti et al., 2003; Pilotti, 2005; Sanderson, 2005) that cause fresh infection of roots besides spread through root to root contact among trees (Turner, 1965; Ariffin et al., 2000). Hennessy and Daly (2007) reported that at the advanced stage, the disease could be generally identified by the distinctive fruiting body or bracket, which grows on the trunk of infected plants. Brackets emerge once the infection has spread significantly through the plant, resulting in its death. The spores incorporated into the soil, germinate and then the hyphae grow over the roots. The fungus moved from the roots to the woody trunk tissue where it destroyed the xylem (water conducting tissue). Primary symptoms that observed included a mild to severe wilting of leaves and die-back of some branches. Infected trees died gradually depending on the environmental conditions (Schwarze and Ferner, 2003).

Recently, Vinayaka and Prathibha (2013) described the disease symptoms that are characterized by yellowing of the lower leaves and decay/ death of fine roots. Bleeding patches appeared at the base of the stem near the ground level with lesions gradually extending upwards, leaves drooping followed by button shedding and barren nuts. They have also reported stem decay that traverse upwards, outer leaf whoring and drooping off followed by spindle leaf drooping except erect and healthy two/three leaves that also fall off leaving the decapitated stem finally.

Isolation and establishment of pure culture

Venkatarayan (1936) reported that G. lucidum grew well on malt agar medium. Malt extract medium also supported the good growth of G. lucidum (Adaskaveg and Gilbertson 1987; Shukla and Uniyal 1989; Biley et al., 2000; Lomberh et al., 2002). Potato Dextrose Agar has been found good medium except for a slightly more time required for growth (Booth 1971; Biley et al., 2000). Sharma and Thakur (2010) reported that radial growth of G. lucidum was higher in malt extract agar added with linseed extract medium. Palanna et al., (2009) reported isolation of Ganoderma from sporophore and diseased root bits that were found good inoculum sources.
**Identification of species of *Ganoderma***

The taxonomy of Basidiomycota is traditionally has been based on the morphological features of the basidiocarps. Identification based on these basidiocarp features, however, is prone to problems such as absence of basidiocarps during certain times of the year their morphological plasticity and presence of cryptic species (Moncalvo and Ryvarden, 1997; Gottlieb and Wright, 1999a).

For these reasons, contemporary taxonomy and identification of *Ganoderma* species employ morphological studies, mating tests, analyses of biochemical and DNA sequence, or combinations. The fungus *Ganoderma lucidum* was first described under the name *Fomes lucidus* (Leys.) Fr. and Butler described the fungus in detail in 1909. The following are considered as synonyms: *Fomes lucidus* (Eys.) Fr., *P. amboinensis* (Lam.), *P. lucidus* Fr. *P. amboinensis* Fr., *P. egregious* Masse, *G. amboinensis* (Am.) Pat., *G. resinaceum* (Butler and Bisby, 1931), *G. sessile* Murrill, *P. fulvellus* Bres., *P. resinosus* Schraeder, *P. curtisil* (Berk.) Murill, and *G. mangiferae* (Lev.) (Van Overem, 1926).

Taxonomically, *Ganoderma* belongs to the Phylum: Basidiomycota; Order: Aphyllophorales and Family: Ganodermataceae (Alexopoulos et al., 1996) the family: Polyporaceae (Ganodermataceae) and the genus: *Ganoderma* (Wasser and Weis, 1999). In all, 219 species within the family have been assigned to the genus *Ganoderma*, of which *G. lucidum* (W. Curt.: Fr.) P. Karsten is the type species (Moncalvo, 2000).

The family, Polyporaceae is classified on the basis of tiny holes on the underside of the fruiting body which contain reproductive spores. They have a woody or leathery feel and the presence of these pores are obvious characteristics that distinguish polypores from other common types of mushrooms. Polypores, like other fungi, grew on wood as an expansive network of microscopic tubes known as mycelium. They degrade the wood over time and produce a fruiting body (conk) on the surface of the wood. *Ganoderma* species are among those fungi that can thrive under hot and humid conditions and are usually found in subtropical and tropical regions (Moncalvo and Ryvarden, 1997).

The *Ganoderma* was further subdivided into two subgenera on the basis of presence of laccate (*G. lucidum* complex) and non-laccate (*G. applanatum*) (Moncalvo and Ryvarden, 1997). More than 250 *Ganoderma* species have been described worldwide, and most of these descriptions have been based on only pleomorphic characters and therefore uncertainty exists about the taxonomic status (Ryvarden, 1994).

Such taxonomic problems lead to the misuse of names, absence of type specimens, the large number of synonymies and differences in morphological characters. Due to such problems, Moncalvo et al., 1995a, b; Smith and Sivasithamparam, 2000a; Gottlieb et al., 2000; Hong and Jung, (2004) proposed methods to determine the identity of *Ganoderma* species including cultural characters, sexual compatibility studies, isozyme studies and DNA based techniques.

**Cultural characteristics**

In addition to basidiocarp morphology, cultural characteristics such as chlamydospore production, growth rate and thermophily have been used to differentiate *Ganoderma* species (Adaskaveg and Gilbertson, 1986, 1989). Hyphal structures in culture such as generative hyphae with clamp connections, fibre or skeletal hyphae, stag-horn hyphae, cuticular cells and vesicles, and hyphal...
rosettes as well as chlamydospores production besides growth rate and thermophily of the cultures distinguished *Ganoderma* species (Seo and Kirk, 2000). Rajendran et al., (2013) reported that most of the *Ganoderma* isolates produced a dense mycelial growth on PDA medium and a few isolates showed sparse mycelial growth. Most of the isolates appeared white in the initial stage of growth and later the colony colour changed from white to pale yellow or light brown.

**Morphological characteristics**

*Ganoderma* is morphologically most complex genus of family *Ganodermataceae* of Aphyllophorales. Different characteristics, such as shape and colour (red, black, blue/green, white, yellow and purple) of fruit body, host specificity and geographical origin, were used to identify individual members of the species. Fruiting bodies of *G. lucidum* were stipitate, dimidiate or reniform and rarely suborbicular, thick, corky, yellowish in margin which turn brownish with shining laccate on the surface (Wong et al., 2012).

The shape and size of the basidiospores and the cuticle cells have been considered as the two most important characters in the genus *Ganoderma*. All *Ganoderma* species lack cystidia, have echinulate basidiospores and often cause white rot in the substrate/host (Steyaert, 1972). Basidiospores of *G. lucidum* were brown, ovate with a rounded base and truncate to narrowly rounded apex.

The epispores were smooth and the endospores were rough with large central gutta (Chang and Miles, 2004). Surface of the basidiospores were dimpled and the wall composed several layers (Furtado, 1962; Pegler and Young, 1973). Mims and Seabury (1989) reported that the two walls of the basidiospores were separated by columns and the spore surface appeared punctate.

**Molecular characteristics**

Molecular based methodologies adopted for identifying *Ganoderma* species include isozyme variation (Royse and Mary 1982), recombinant DNA (rDNA) sequencing (Moncalvo et al., 1995b; Gottlieb et al., 2000), random amplified polymorphic DNA (Wang et al., 2003), internal transcribed spacer (ITS) sequences (Hseu et al., 1996; Soko et al., 1999), sequence-related amplified polymorphism (SRAP) (Sun et al., 2006), enterobacterial repetitive intergenic consensus (ERIC) elements and amplified fragment length polymorphism (AFLP) (Qi et al., 2003; Zheng et al., 2009).

Hseu et al., (1996) used the PCR with arbitrary primers to randomly amplify DNA fragments of 36 isolates of the species complex, *G. lucidum*. Results showed that data from RAPD did not distinguish the same clades as ITS data did. However, it resolved several robust terminal clades containing putatively conspecific isolates, suggesting that RAPDs might be helpful for systematics at the lower taxonomic levels that were unresolved by ITS sequence data. Thus, it was concluded that ITS sequences could be used to identify isolates of the *G. lucidum* complex, whereas RAPDs be used to differentiate between isolates having identical ITS sequences.

Moncalvo et al., (1995a) used sequencing of ITS region and domain D2 of 25s rDNA of *Ganoderma* and identified four phylogenetically related clusters of 14 strains of laccate *Ganoderma* species. Moncalvo et al., (1995b) compared the molecular data (ITS and D-2) of 29 isolates of *G. lucidum* collected from temperate and subtropical areas and identified six monophyletic groups of laccate *Ganoderma* species. Five of the six groups, collectively comprising 26 strains, contained at least one strain that labelled as *G. lucidum*.
Gottlieb et al., (2000) adopted rDNA analysis (ITS I and II of 5.8S rDNA) to identify South American isolates of Ganoderma and Elfvingia and found molecular and morphological agreement at the subgeneric level, however this relationship was difficult to visualize at the species level. Singh et al., (2003) characterized 61 accessions using DNA finger printing technique and RAPD/AFLP analysis revealed highly significant genetic variability among G. lucidum isolates collected from coconut gardens in Coimbatore. Phylogenetic analysis based on RAPD profiles and direct sequencing of 5.8S rRNA gene region revealed intergeneric interspecific and intra specific variations in Volvariella, Lentinula, Ganoderma and Calocybe groups of the accessions. G. lucidum exhibited intraspecific variability and isolates could be distinguished into three different clades. Similar molecular studies on G. lucidum through RAPD analysis were conducted by various workers, namely Miller et al., (1999), Mandal et al., (2003), Postnova and Skolotneva (2009), Ghazala et al., (2010) and Rolim et al., 2011. Hong and Jung (2004) used a newer molecular technique based on nearly complete sequencing and secondary structures of mitochondrial small sub-unit ribosomal DNA (mt SSU rDNA) of Ganoderma species, which was more informative and superior technique to 5.8S rDNA technique. Wang and Yao (2005) also tried ITS heterogeneity at the intrastrainal level in Ganoderma.

Tang et al., (2005) conducted preliminary studies on genetic diversity among ten strains of Ganoderma by using RAPD and esterase enzyme analysis. RAPD analysis clustered the strains into three groups. The first group included G. resinaceum, G. lucidum cv. xinzhou and G. lucidum; the second group included G. subamboinense and G. sinense and the third group included G. applanatum. However, esterase isoenzyme analysis divided the strains into two groups. A new novel marker, Sequence Related Amplified Polymorphism (SRAP) was used by Sun et al., (2006) for analyzing molecular diversity in Ganoderma population consisting of 31 accessions including commercial cultivars and wild varieties. Out of 95 combinations of primers 50 turned out to be polymorphic and 85 polymorphic bands were obtained by using six combinations. Based on the appearances of markers, the genetic similarity coefficients were calculated and genetic variations were observed among them. Significant differences were brought forth between G. lucidum and G. sinense and also found that G. lucidum in China was different from G. lucidum in Yugoslavia.

Karthikeyan et al., (2007) used RAPD to determine the genetic relatedness within and between G. lucidum isolates from infected coconut palm. Dendrograms from cluster analysis based on unweighted pair–group method using arithmetic means (UPGMA) of RAPD data analysis of ten random primers showed variations of banding patterns within and between the isolates from coconut and other hosts, indicating that they were genetically heterogeneous. Rai et al., (2007) clearly distinguished Tyromyces, Ganoderma and Trametes from each other through direct sequencing using ITS-I and ITS-4 primers. Su et al., (2007) developed a strain Specific Sequence Characterized Amplified Region (SCAR) marker for authentication of G. lucidum. One fragment unique to strain 9 of G. lucidum was identified by Inter Simple Sequence Repeats (ISSR) and then sequenced. Based on that specific fragment, one SCAR primer pair was designed to amplify a 612bp DNA fragment within the sequenced region. Diagnostic PCR was performed and results showed that this SCAR marker can clearly distinguish strain No. 9 from other related G. lucidum strains.
Zheng et al., (2009) used Amplified Fragment Length Polymorphism (AFLP) having 14 primer combinations and internal transcribed spacer (ITS) PCR-RFLP in a comparative study, designed to investigate the genetic relations of closely related Ganoderma strains at molecular level. The analysis of 37 Ganoderma strains showed that there were 177 polymorphic AFLP markers and 12 ITS PCR-RFLP markers and all accessions could be uniquely identified. Among the Ganoderma accessions, similarity coefficients ranged from 0.07692 to 0.99194 in AFLP. The Ganoderma strains formed a tight cluster of nine groups in AFLP whereas seven groups in ITS PCR-RFLP. The cluster analysis revealed that the taxonomical system of subgenus Ganoderma is composed of Sect. Ganoderma and Sect. Phaeonema.

Rueankeaw and Danuwat (2010) employed ISSR technique to determine the phylogenetic relationships among nine G. lucidum (MG1-MG 9) strains using 16 primers. The 11 primers gave 21 specific amplified fragments in 7 out of 9 strains tested, which could be use as specific markers for strain identification. The strain MG3 had more distinctive morphology and more specific fragment than the others strains. The results of the phylogenetic tree indicated that the strains MG3 and MG4 were genetically distant from the other strains. This suggested ISSR technique as a useful method for polymorphic identification of G. lucidum strains, since it revealed a high degree of polymorphism. Park et al., (2012) analysed the ITS rDNA region and partial beta tubulin gene sequences of Ganoderma species in order to clarify their genetic relationships and found that Korean G. lucidum strains were different from those of China, Taiwan and Canada.

Nusaibah et al., (2007) reported that genetic variation among 44 isolates of Ganoderma sp. isolated from the basidiocarps of four different hosts (oil palm, rubber, tea, and forest trees) collected from selected areas of Peninsular Malaysia. Restriction Fragment Length Polymorphism (RFLP) technique, using ITS1 and ITS4 primers, the isolates from tea and rubber were more closely related compared to oil palm and forest trees. Similarly, the Ganoderma isolates from the same host were also clustered together, and three species identified were G. boninense (from oil palm and coconut stumps), G. philippii (rubber) and G. australe (forest trees).

**Virulence of Ganoderma isolates**

Various methods of artificial inoculation of oil palm seedlings had been carried out. Lim et al., (1992) successfully inoculated injured roots of healthy 15-month-old palms by placing wheat-oat medium inoculated with Ganoderma. Idris et al., (2004) employed root inoculation method where primary roots of oil palm seedlings in polybag were exposed and inserted into test tubes containing various Ganoderma spp. isolates grown in POPW medium (mixture of paddy, oil palm wood sawdust, supplemented with sucrose, ammonium sulphate, calcium sulphate, and bacto peptone). Of a total of 344 isolates tested, 304 isolates were found pathogenic and 40 isolates were non-pathogenic.

Khairudin et al., (1991) reported 100% success in infecting oil palm by wrapping bare roots of seedlings over rubber wood blocks (RWB) (6 x 6 x 12 cm or 432 cm3) pre-inoculated with Ganoderma boninense. In Indonesia, inoculating seedlings using oil palm and rubber wood blocks in a range of sizes (Rees et al., 2007) as the substrates had been reported. However, Ganoderma-inoculated RWB still remains the most effective option for artificial inoculation of Ganoderma spp. to screen for Ganoderma tolerance and to study various control
measures in the nursery (Breton et al., 2006 and Nur Ain Izzati and Abdullah, 2008). Among the nine Ganoderma isolates of coconut tested for the pathogenicity on Tiptur tall coconut seedlings, only two isolates (G13 and G14) were pathogenic to coconut seedlings. These two isolates were isolated from the root samples and were identified as Ganoderma applanatum based on colony morphology and mycelial characters (Palanna et al., 2009).

Breton et al., (2006) observed that artificial inoculation methods of Ganoderma boninense isolates to oil palm seedlings were one of the main parameters affecting the level of disease severity and aggressiveness of isolates collected from three different estates in Indonesia.

They have suggested investigating growth rates of different G. boninense isolates under in-vitro conditions and variations in the degree of virulence in oil palm seedlings. Virulence of 10 Ganoderma isolates of arecanut tested under pot culture revealed that isolate AG9, recorded maximum disease severity index (75 DSI) nine months after inoculation followed by AG22 and AG4 which accounted 68.8 DSI each (Palanna et al., 2018)

**Disease management**

Basically, the Ganoderma is a soil-borne pathogen and it survives well in the soil for a long time. The incubation period of this disease has been determined to be several years (Turner, 1981). The visible disease symptoms appear at a very late stage of infection when more than half of the root tissues have been decayed. Basal stem rot disease could be contained by management practices with the integration of cultural, chemical and biological methods if the disease is detected in the early stages.

**Early detection**

BSR is like a ‘cancer’ in palms especially in coconut, arecanut as well as in oil palms, and it is very difficult to detect at the early stages of the disease. The limiting factor in the control of BSR is the lack of early disease detection. Some conventional diagnostic tools have been developed for early diagnosis of BSR such as (i) the colorimetric method, using Ethylene Diamine-Tetraacetic Acid (EDTA), which is used to detect G. lucidum in coconut (Natarajan et al., 1986), (ii) semi-selective media for Ganoderma cultures from oil palms (Darus et al., 1993), and (iii) Ganoderma-selective media (GSM) which detect the pathogen from any infected tissues. According to Ariffin et al., (1996), GSM detected Ganoderma in oil palms that were infected but not shown any external symptoms. Due to the low accuracy these conventional methods have not been recommended.

Currently, advance molecular techniques have been innovated with more accuracy of detection and fungal identification. Two diagnostic methods used to detect Ganoderma, such as (i) the use of polyclonal antibodies (PAbs) in the pathogen using enzyme-linked immunosorbent assay (ELISA), and (ii) the use of polymerase chain reaction (PCR) methods using specific deoxyribose nucleic acid sequences of the pathogen (Kandan et al., 2009; Utomo and Niepold, 2000; Priestley et al., 1994; Fox and Hahne, 1989). This laboratory based techniques were suitable for small scale samples which were not applied for field condition. Currently, development of device system in agriculture technology such as remote sense system or e-nose system is being used to detect real-time disease monitoring. However, the results showed that the e-nose system could discriminate the Ganoderma infected plant in field condition but not able
to detect the stages of infection levels or early infection of the disease (Santoso et al., 2011; Azahar et al., 2011; Markom et al., 2009). Several studies have shown that fluorescence or spectroscopic imaging (Chaerly et al., 2007); infrared spectroscopy (Spinelli et al., 2006); visible/ multiband spectroscopy (Choi et al., 2004) were used to detect any biotic or abiotic stress related symptoms in plants. These techniques could be developed to detect BRS or *Ganoderma* infection in oil palm.

For detecting the presence of *G. lucidum* inoculum in coconut gardens, various plant species were tested of which Subabul (*Leucaena leucocephala*) and *Glyricidia maculata* were very useful as indicator plants since these plants showed natural infection under field conditions at least six months earlier to infection on coconut palms (Anon., 1989). Karthikeyan, *et al.*, (2006) reported that molecular and immunological methods for detecting the *Ganoderma* disease of coconut. Rajendran, *et al.*, (2014) reported PCR as a new technique that has gained broad acceptance very quickly in many areas of science and application of the two Gan1 and Gan2 primers generated from the ITS1 sequence proved useful for the specific detection of plant pathogenic *Ganoderma* isolates.

**In vitro evaluation of antagonists against *Ganoderma***

Dharmapatra *et al.*, (1989) reported that *Penicillium citrinum* inhibited the growth of the pathogen and formed a zone of inhibition on the agar media. *Trichoderma harzianum* BIO - 1 as well as BIO - 2 and *T. viride* not only repressed the growth of the pathogen but also caused lysis of the hyphae, and the colony was totally overgrown by the antagonists.

Bhansali (2003) reported that in dual-culture technique *Trichoderma* inhibited the mycelial growth of *Ganoderma lucidum* on potato dextrose agar under *in vitro* conditions. Among the three strains of *T. harzianum* and *T. viride* inhibited the maximum mycelial growth by acting as antagonists to *G. lucidum*. Iyer *et al.*, (2004) reported that four fungal cultures viz., an unidentified sterile white fungus (77.80), *Trichoderma harzianum* (72.20), *T. viride* (62.0) and *Pencillium* sp. (42.0) were found to be inhibitory on the mycelial growth of pathogen at 96hrs. Srinivasulu *et al.*, (2004) reported that *Trichoderma viride* completely inhibited *Ganoderma applanatum* and *Ganoderma lucidum*. Reports of CPCRI revealed that the culture filtrates of *Trichoderma harzianum* and *Bacillus amyloliquefaciens* showed antagonistic property against *Ganoderma* . Culture filtrate of *T. harzianum* inhibited the mycelial growth of *Ganoderma* (54 per cent), while culture filtrate of *B. amyloliquefaciens* showed 80 per cent inhibition. Anon. (2007) revealed that among the three *Trichoderma* spp. tested, the maximum inhibitory effect on the pathogen was exerted by *T. virens* (74 %), followed by *T. viride* (69 %) and *T. harzianum* (58.5 %). Among the bacterial antagonists tested, the maximum inhibition was produced by *Pseudomonas fluorescens* 1 (Pf1) (83.1 %), followed by *Bacillus subtilis* (Bs1) (83.0 %), *P. fluorescens* 2 (Pf2) (50.5 %) and *Bacillus* sp2 (Bs2) (43.0 %).

Among the 17 bio-control agents screened, native *Trichoderma* sp. (V2) recorded minimum radial growth of 1.72 cm by exerting 81 per cent reduction over control, it was followed by *Trichoderma* sp. (12a), that recorded 2.30 cm radial growth with 74 per cent reduction over control (Palanna *et al.*, 2013). Rajendran *et al.*, (2007) reported that fifty five endophytic bacterial strains isolated from coconut roots of different regions, isolate EPC5 (Endophytes coconut), EPC8,
EPC15, EPC29, EPC52 and Pf1 (Plant growth promoting rhizobacteria) promoted the rice seedling growth in roll towel and pot culture method. EPC5 (Plant growth promoting endophytic bacteria), Pf1 and Trichoderma viride (Plant growth promoting fungus) effectively inhibited the G. lucidum growth in vitro. Chakrabarty and Ray (2007) reported that dual culture studies revealed that the three fungal cultures viz., Trichoderma harzianum (63.99 %), Trichoderma viride (66.55 %) and Gliocladium virens (62.12 %) have inhibitory effect on the mycelial growth of the pathogen after 96 hours of incubation.

In vitro studies have shown that the fungi Trichoderma spp., Aspergillus spp., and Penicillium spp. antagonistic towards Ganoderma (Bruce and Highley, 1991; Badalyan et al., 2004) and were found good bio-control agents against Ganoderma (Susanto et al., 2005; Bruce and Highley, 1991).

The fungal bioagent Trichoderma koningi (Tk-1) recorded maximum (59.86) inhibition of Ganoderma Spp. Causing Foot Rot of Areacanut and remained on par with T. viride (Tv-GKVK2). However, the volatile metabolites of T. viride (Tv - NBAIR) recorded maximum (71.07%) inhibition. Among the bacterial bio-agents, B. amyloliquefaciens (Ba-43) recorded maximum (63.93 %) inhibition followed by B. subtilis (Bs-P22) which accounted 59.27 per cent inhibition over control in dual culture technique. When the effect of volatile metabolites of bacterial bioagents considered, B. subtilis (Bs-P22) recorded maximum (70.53 %) inhibition followed by B. subtilis (Bs-P42) and P. putida (Pp-1) which accounted 70.00 and 67.07 per cent inhibition over control by producing volatile metabolites. The volatile metabolites of Fungal and bacterial bioagents are found to be more promising in inhibition of Ganoderma Spp. Causing Foot Rot of Areacanut compared to the dual culture technique under in vitro (Palanna et al., 2017a).

**In vitro evaluation of botanicals against Ganoderma**

The plants and its derivatives are of great use in agriculture, public health, medicines, cosmetics, etc. Plant extracts are effective against plant pathogens as they have unique antimicrobial properties that act in a holistic manner due to presence of certain secondary metabolites, viz., alkaloids, terpenoids, glycosides and phenolic acids (Srivastava et al., 1994; Singh et al., 1999). Garlic extract of 10 per cent concentration completely arrested the growth of T. viride, T. harzianum and T. hamatum and both the species of G. lucidum and G. applanatum (Srinivasalu et al., 2002). Neem cake extract, banana rhizome extract and Tephrosia purpurea extract gave 100, 86 and 54 per cent inhibition respectively (Bhaskaran et al., 1983).

Kharkwal et al., (2012) determined the antifungal activity of the dealcoholized extract of the leaves of Clerodendron infortunatum Retz. against four fungal organisms i.e. A.niger, P. frequentance, P. notataum and B. cinera. Bhardwaj (2012) carried out test of aqueous extract of twenty plants for their antifungal activity against Fusarium solani, the causal organism of dry rot disease of potato. Among 10 botanicals tested under laboratory conditions, only Glyricidia was found inhibitory against G. applanatum, by recording radial growth of 5.4 cm as against 9.0 cm in control (Palanna et al., 2013). Garlic was found to be fungitoxic to a number of plant pathogen (Iyer et al., 2004, Gowda and Nambiar, 2006, Chakrabarty et al., 2013). Crude extract of different plant parts of Solanum nigrum obtained using solvents viz., petroleum ether, chloroform, acetone, ethanol and methanol.
showed that leaf aqueous extract was more effective against all the microbes tested (Ramya et al., 2012).

Antifungal activity of a perennial aquatic herb (*Eichornia crassipes*) was tested against *Ganoderma lucidum*, basal stem rot pathogen of coconut at two concentrations (150 mg/ml and 300 mg/ml). Among these concentrations, 300mg/ml was found to be most effective in inhibition of pathogen (Deepatharshini and Ananthi, 2015).

**In vitro evaluation of chemicals against Ganoderma**

Results of experiments carried out in CPCRI, Kasargod revealed that six fungicides viz., Calixin (Tridemorph 80 % WP), Bavistin (Carbendazim 50 % WP), Captra (Captan 50 % WP), Companion (Carbendazim 12 % + Mancozeb 63 % WP), Diodine (65 % WP), Matco-8-64 (Metalaxyl 8 % + Mancozeb 64 %) and Indofil-M-45 (Mancozeb 75% WP) tested for their efficacy on *Ganoderma* isolates, Tridemorph 80 % EC and Companion (Carbendazim 12 % + Mancozeb 63 % WP) proved to be the most effective (Anon, 2000). Among the fungicides tested, copper oxychloride (0.3 %), Bordeaux mixture (1 %), Calixin (0.1 %) and Contaf (0.1 %) exerted complete inhibition (Anon., 2013).

Watanabe et al., (1987) reported that, the mycelial weight of eight out of nine isolates of *Trichoderma* sp. and *Glicladium virens* increased in media supplemented with 2g/l N supplied as NH4 Cl, NaNo3 and a commercial 20:20:20 fertilizer. Neelamegan (1992) observed better growth of *T. viride* when ammonical form of N was incorporated in the medium. The addition of ammonium sulphate was supportive and stimulatory to the growth of *T. harzianum* (Sharma and Mishra., 1995). ZnSo4.7H2O (1 %) and found to inhibitory to *Ganoderma* sp. but not to *T. viride*, *T. harzianum* and *T. hamatum* Srinivasalu et al., (2002). Jaganthan and Ramasamy (1975) stated that ZnSo4 found to inhibit the *G. lucidum* and *G. applanatum* completely and significantly hampered the growth of *Trichoderma* sp. The manganese sulphate when applied @ 227g/palm/year reduced the intensity of BSR disease in coconut.

Palanna et al., (2005b) reported that manganese sulphate, magnesium sulphate, ammonium molybdate, calcium sulphate and ferrous sulphate are found to be supportive for the growth of *T. viride* under *in vitro* conditions, whereas copper sulphate and sodium borate exerted 100 per cent inhibitory effect. Sulkla and Mishra (1970) reported that growth of *T. viride* was significantly increased by Na, K and Mg salts compared to medium without salts. Srinivasalu et al., (2002) reported that Bordeaux mixture (1 %), Copper oxychloride (0.3 %) Bitertanol (0.1 %), Tridomorph (0.1 %), Hexaconazoal (0.1 %) and Traidemifon (0.1 %) were found completely inhibiting the growth of *G. lucidum*, *G. applanatum* and three species of *Trichoderma*.

**Integrated disease management**

Basal stem rot disease caused by *Ganoderma* considered most destructive disease of palms. Resistant mycelium, basidiospores, chlamydospores, and pseudosclerotia present in *G. boninense* influence the control of *Ganoderma* (Susanto et al., 2005; Naher et al., 2012b). Also, a dead or felled oil palm could spread the disease by spore or root contact (Sanderson, 2005; Paterson, 2007). Hence, no satisfactory method exists to control BSR in the field condition. However, studies using various methods such as the trenching, replanting techniques and chemical control had given different paths with great
potential. Digging trenches around infected palms to prevent mycelium spread by root contact with neighboring healthy palms has been recommended as a management practice. Sanitation during replanting regarded as an important practice for controlling BSR. The result showed that this method could only lower the disease incidence (Singh, 1991). Biological control of BSR disease or Ganoderma spp. achieved with prophylactic application of Trichoderma at early stages of the disease (Abdullah et al., 2003).

Bio control agents like Trichoderma harzianum and T. viride were reported to be antagonistic to Ganoderma lucidum (Gunasekaran et al., 1986 and Baskaran, 1990a). Nur Ain Izzati and Abdullah (2008) reported that disease suppression in Ganoderma infected oil palm seedlings treated with a conidial suspension of Trichoderma harzianum FA 1132 was tested in plant house conditions to determine the effectiveness of the fungus as a biocontrol agent.

The highest efficacy of control was achieved by treatment right after artificial infection; the total number of infected plants was reduced to give the lowest disease severity index (DSI) value of 5.0 per cent, compared to the infected and non-treated control that had the highest DSI of 70.0 per cent. Jayarajan et al., (1987) stated that Neem cake is effective in reducing Ganoderma wilt of coconut. Palanna et al., (2005a) stated that chemical fertilizers like DAP and SSP are supportive for growth and sporulation of Trichoderma viride at lower concentration and reveals that these fertilizers can be included integrated disease management approaches along with Trichoderma.

Palanna et al., (2017) evaluated commonly used agrochemicals in coconut ecosystem on Ganoderma spp. and bio-control agent Trichoderma. Among chemical fertilizers tested against Ganoderma sp. urea recorded maximum (67.34 %) inhibition over control followed by single super phosphate which accounted 53.48 per cent inhibition over control at 1000 ppm. Among micro nutrients borax accounted 100 per cent inhibition over control at all concentrations tested and ZnSo4 accounted 100 per cent inhibition over control at 500 ppm and 1000 ppm concentration. However, on T. viride all chemical fertilizers tested are not found to inhibitory at lower concentration (250 ppm) whereas, at higher concentration (1000 ppm) urea, single super phosphate and muriate of potash are found to be inhibitory except DAP and it was found to be supportive for growth and sporulation of T. viride. Among micro nutrients borax exerted 100 per cent inhibition over control at 500 ppm and 1000 ppm. ZnSo4 and Gypsum recoded 52.53 and 7.42 per cent inhibition over control at 1000 ppm however, MgSo4 was found to be supportive for growth and sporulation of T. viride.

Sampath Kumar and Nambiar (1990) reported that drenching the base of the palm with Captan or Carbendazim at 0.3 per cent concentration was effective in preventing the spread of the disease to the neighbouring palms. Bhaskaran (1993) reported that root treatment with Tridemorph (2ml/100ml) at quarterly interval for one year combined with application of 5kg neem cake per palm per year controlled the basal stem rot of coconut effectively. The chemical treatment (carboxin and quintozene fungicides) showed significant reduction in BSR incidence (George et al., 1996).

Naik (2001) reported the lowest BSR index with the application of Tridemorph root feeding (2 %) + soil drenching (0.3 %) followed by Hexaconazole root feeding (1 %) + soil drenching (0.2 %); soil drenching with
Tridemorph (0.3%) and Hexaconazole (0.2%) compared to root feeding Tridemorph (2%) or Hexaconazole (1%) alone in the gardens. Naik and Venkatesh (2001) reported that use of 2 per cent Tridemorph for root feeding and 0.1 per cent as soil drench in combination with neem-cake application for managing basal stem rot of coconut in Karnataka. In Kerala, soil drenching with copper oxychloride 0.4 per cent at 15 l/tree is generally practiced to combat basal stem rot of coconut. Srinivasalu et al., (2004 b) reported that native bio control agents viz T. viride, T. harzianum, T. hamatum were found to be inhibitory to G. applanatum and G. lucidum. Tridemorph (0.1 %) and Hexaconazole (0.1 %) were found to completely inhibit both G. applanatum and G. lucidum under in vitro condition.

Mohd Farid et al., (2006) reported that the most common practice of rubber growers to combat white root disease was the application of fungicides by means of soil drenching. Several fungicides, such as Hexaconazole, Tridemorph, Propiconazole, Tridemefon, Cyproconazole and Penconazole have shown promise in the control of disease caused by Rigidoporus lignosus. The efficacy of the fungicide treatment, however, reduces with increasing levels of infection (Ismail and Shamsuri, 1998). Fungicides should therefore be applied only on newly infected trees or trees with mild infection levels.

Karthikeyan et al., (2009) reported that in integrated disease management (IDM), fungicide tridemorph treated palms showed low infection level (O.D value) within seven months and T. harzianum and P. fluorescens + T. viride treated palms showed below infection level (OD value) of the disease in eighth month. Combination of T. viride (50g) and neem cake @ 5 kg/palm/year was found to be highly effective in the management of BSR disease of coconut (Srinivasalu et al., 2004).

Bhaskaran et al., (1990) stated that incorporation of organic manures, especially neem cake into the soil and irrigation during summer reduced disease severity. Root treatment of coconut palm infected by Ganoderma lucidum with Tridemorph (2ml/100ml water) at quarterly intervals for one year combined with annual application of 5 kg neem cake/palm reduced disease incidence and increased yields by 132 per cent (Bhaskaran, 1993). Application of Neem cake @ 10 kg/palm/year increased the total population of fungi in rhizosphere and inhibited the growth of G. lucidum (Gunasakaran et al., 1986). Srinivasalu et al., (2001) stated that 50gm T. viride + Neem cake (1kg) per palm per year controlled the linear spread of Ganoderma to the extent (22cm) against 77.6 cm in un-treated palms. The lowest disease index was recorded in treatment with Tridemorph root feeding (2 %) + Soil drenching (0.3 %), followed by Hexaconazole root feeding (1 %)+ soil drenching (Naik, 2001). Karthikeyan et al., 2006, stated that, the mixture of two antagonists (P. fluorescens + T. viride) suppressed Ganoderma disease development in coconut. Tebuconazole 25.9 per cent EC root feeding @1.5 ml in 100 ml water / palm at quarterly interval + Soil application of 5 kg Neemcake enriched with Trichoderma viride / palm /half yearly + Pseudomonas fluorescens (talc formulated) @ 50 g/palm/ half yearly + Soil drenching with 1 per cent BM @ 20 L / Palm half yearly with an increase of 10.57 disease index over pre-treatment which accounted for 76.41 per cent reduction over control and recorded maximum nut yield (51.55 nuts / palm / year) as against 22.66 in control (Palanna and Narendrappa, 2016).

From the previous conversation it is apparent that Ganoderma wilt and Foot rot caused by Ganoderma Spp. in coconut and arecanut respectively are most important diseases limiting production and productivity of palms.
Several researchers worked on identification of potential bio-control agents and promising fungicides and other aspects of the disease. However, more emphasis has to be given on Understanding the mechanism of Ganoderma disease development through histopathological/ histochemical studies; development of molecular techniques to detect disease at incipient stage; influence of cropping system and agronomic practices on disease incidence and Integrated disease management of Ganoderma wilt through large scale demonstration to mitigate the disease there by production and productivity of the palms could be enhanced.

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