

Review Article

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The Feasibility of Producing Oil Palm with Altered Lignin Content to Control *Ganoderma* Disease

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Abstract

Oil palm is a major crop which is grown for the production of vegetable oil used in foods, cosmetics and biodiesel. The palm is of major economic importance in southeast Asia where it is grown extensively in Malaysia and Indonesia. There is concern about *Ganoderma* rots of oil palm which need to be controlled to prevent major infection. However, the basic mechanism of white-rot infection has been ignored. White rot implies that fungi attack the lignin component of woody tissue leaving the white cellulose exposed. The fungus grows within palms by utilizing cellulose in the tree. By altering the lignin fraction of oil palm losses may be reduced. Methods for altering lignin in plants are reviewed here to indicate how similar transformations could be attempted for oil palm. In addition, progress in transforming oil palm is described. Lignin is extremely complex and this may explain why it has not been studied in oil palm. Some crops transformed with *Bacillus thuringiensis* toxin genes have increased lignin and modified oil palm have been produced using *B. thuringiensis* genes. These require to be tested for lignin concentration and structure. The nomenclature of the disease organism is discussed. The prospects for altered lignin oil palm are considered herein.

Introduction

Palm oil is a major commodity (Corley and Tinker, 2003). The product is used worldwide and is included in c. 30% of foods, pharmaceuticals and cosmetics. Biodiesel is increasing in significance, concomitant with technologies to convert palm oil to the fuel (Kalam and Masjuki, 2002).

Oil palm is subject to various diseases with root, basal stem and upper stem rots caused by *Ganoderma*

being of particular significance to, for example, Malaysia (Paterson et al., 2008). Plant disease is important particularly where monocultures are involved as is the case for oil palm (Paterson, 2007). However, research involving the fundamental biochemical processes has been neglected in favour of that concerning the method of spread between palms and the genetic variability of *Ganoderma*. In particular, the fungus is considered to be a white-rot fungus implying that it can attack selectively lignin and leave the white cellulose exposed. In a major review, Paterson (2007) emphasized that this aspect has been ignored and suggested briefly that altering lignin in oil palm may be beneficial.

The number of species involved in the disease has not been resolved satisfactorily. Ryvardeen (1994) proposed, somewhat facetiously, that new species of *Ganoderma* should not be described until 2005 because of the confusion in the taxonomy (Seo and Kirk, 2000). The organism from Malaysia was described originally as *Ganoderma lucidum*, although it was considered subsequently that it would be unlikely to be a single species. Ho and Nawawi (1985) stated that all strains from peninsular Malaysia were *Ganoderma boninense*. However, other authors suggested in 1990 that (i) alternatives may be involved and (ii) *Ganoderma tornatum* was also present. Approximately 5 years later, many of the researchers on *Ganoderma* had not decided on a particular species name for the disease fungus. Flood et al. (2000) used *G. boninense* for oil palm disease strains from Indonesia, Malaysia and Papua New Guinea. Nevertheless, Corley and Tinker (2003) mentioned that *Ganoderma miniatocinctum*, *Ganoderma zonatum* and *G. tornatum* were associated with the disease and reverted to using the genus name for all the taxa involved. Finally, Ali et al. (2004) considered *G. boninense*, *G. miniatocinctum* and *G. tornatum* in their work.

Hence, for the sake of clarity, the genus name only will be used in the present review as used by 'most authors' (Corley and Tinker, 2003; Paterson, 2007).

Oil palms are grown in tropical areas of Asia, Africa and America, and the oil palm yields per hectare are the highest of all oil crops (Corley and Tinker, 2003). However, Malaysia and Indonesia provide most of the oil for international trade. Worldwide production rose from 2.2 million tonnes of palm oil in 1972 to 21 million tonnes in 2000. The largest changes have been from Deli *dura* palms to *tenera* palms, although the genetic basis of the industry is remarkably narrow. It is predicted that South America will grow rapidly, as will other Asian countries of the equatorial South East (i.e. Philippines, Thailand and Papua New Guinea). There are concerns that palm oil may be falling behind in the technological race of ever higher yields. On the other hand, genetically manipulated crops have issues of acceptability particularly in Europe and this may affect palm oil, although such technological processes have not been undertaken in oil palm to the extent of other crops. Inevitably, research will be even more important to the future of oil palm.

Transgenic technology offers the opportunity to develop better crops beyond species boundaries with unprecedented power and precision. Also, the procedures extend greatly the understanding of crop biology and provide better methods to improve crops. Approximately 114 million hectares of transgenic crops are grown worldwide (<http://www.isaaa.org>, 13 February 2009). The most important transgenic crops grown commercially are corn/maize, cotton, potato, canola/rapeseed, soybean, squash and papaya. Transgenic soybean and corn are first and second, respectively in the global area planted in transgenic crops. Intensive and advanced biotechnological research on local crops is being undertaken in 5 of the 10 ASEAN countries: Malaysia, Singapore, Thailand, the Philippines and Indonesia. Malaysia has not approved any field trial for commercial transgenic crops as of 2002. Field trials of *Papaya ring spot virus*-resistant papaya and tungro-resistant rice are expected in the near future after enough seeds have been acquired and approval by the Genetic Modification Advisory Committee has been secured. However, Indonesia is reported to have obtained commercial field release status for *Bt* cotton (Hashim et al., 2002).

The purposes of this paper on *Ganoderma* disease of oil palm are to indicate (i) what is known about oil palm biodegradation and (ii) the constraints and potential to produce oil palm with resistant lignin. A review of *Ganoderma* on oil palm is provided by Paterson (2007).

Oil Palm

The palms (*Arecaceae*) form a distinct group of plants amongst monocotyledons. The genus *Elaeis* is derived from the Greek 'elaion' meaning oil and the specific name *guineensis* is attributable to the assumed origin of Guinea. There are three accepted species, *Elaeis*

guineensis, *Elaeis oleifera* and *Elaeis adora*, and the macromolecular composition of these and susceptibility to *Ganoderma* needs to be determined accurately in relation to the present topic. Molecular markers have indicated that *E. adora* can be included within the genus, although it has considerable difference from the other two. Idris et al. (2002) and Durand-Gasselin et al. (2005) observed the high susceptibility of Deli, and the low susceptibility of African oil palm. However, Durand-Gasselin et al. (2005) suggested there was insufficient evidence to support the hypothesis of a difference between *E. guineensis* and *E. oleifera*. Furthermore, investigations into these characters of *E. guineensis* from Madagascar would be worth undertaking as these palms appear to be somewhat distinct, and variation within the species also requires to be further investigated in relation to lignin and biodegradation.

The plants representing the genus have 16 pairs of chromosomes (diploid number, $2n = 32$). There appear to be three groups of chromosomes (one long pair, eight medium length pairs and seven short pairs). Furthermore, fluorescent labelling distinguishes *E. oleifera* chromosomes in crosses between that and *E. guineensis*. Hybrids between the two species have been made frequently.

Ganoderma disease

Interestingly, the reports until World War II indicated that oil palm was free of serious diseases. There have been severe or devastating outbreaks in various parts of the world as the crop area has expanded. The seriousness of the disease, in the case of *Ganoderma*, appears to have increased dramatically over a short period (Paterson, 2007). Indeed, the disease is becoming increasingly prevalent with even younger palms being affected. *Ganoderma* rots have been associated with old and replanted areas of Asia.

There is the possibility of finding resistance to *Ganoderma*, and producing high lignin oil palm is an obvious approach, especially when the white-rot mode of attack is acknowledged fully (Paterson, 2007). Breeding for resistance has indicated that it has potential in controlling *Ganoderma* basal stem rot (BSR), especially when integrated with other approaches (see Paterson et al., 2000). Related to this, differences in incidence between (i) West African and Deli material, (ii) families and (iii) clones have been observed. *In vitro* inoculation methods revealed differences in susceptibility although none were observed in a nursery trial. Interestingly, infection of *Fusarium oxysporum* into oil palm was considered to be impeded by lignification (Corley and Tinker, 2003), hence high lignin oil palm may also assist in controlling *Fusarium* wilts.

We are surprised that knowledge of the *Ganoderma* disease is often anecdotal and several aspects remain obscure despite collaborative research (e.g. Flood et al., 2000; Bridge and Utomo, 2005). The focus of research was concerned with (i) between palm spread of the fungus and (ii) examining the nucleic acids of

Ganoderma. An apparent misconception is that spread is via roots and not basidiospores. However, spread within the oil palm is by growth primarily on macromolecules (e.g. starch, cellulose) and the possibility of spread between palms by hyphal fragments from infected trees remains unreported. The basic method of attack of the oil palm, i.e. white rot, has been ignored (Paterson, 2007). Incidentally, the disease of fatal yellowing or lethal bud rot is interesting and may be caused by *Thielaviopsis paradoxa*. This fungus attacks non-lignified tissue and provides a useful comparison to *Ganoderma*. In addition, the teleomorphic stage *Ceratocystis paradoxa* causes the disease dry basal rot. The use of the term 'dry' is interesting here and has been misused to describe *Ganoderma* rots (Paterson, 2007). The term 'dry stem rot' (Bridge and Uto-mo, 2005) also appears to be inaccurate.

Much more basic information is required about the macromolecular nature of oil palm (cellulose, lignin, starch) and how these components are decayed by *Ganoderma*. This leads to the possibility of reducing decay by inhibiting the enzymes involved (Paterson et al., 2008). Furthermore, oil palm with altered lignin would possibly be more resistant to decay. Time scales to see an improvement in the plantation may be medium to long term, although immediate action can be taken, for example, wounds in the oil palm could be sealed as this will reduce *Ganoderma* from attacking the plant. Tree surgery followed by painting with coal tar to protect the wound can be reintroduced (Zaiton, 2006). The costs of doing this may be justified when the price of palm oil is high.

Oil Palm Composition

The amount of information on this subject is remarkably low with the exception of Sun and Tomkinson (2001). These data are crucial for a complete understanding of how oil palm is decayed. Oil palm trunk fibre had the following chemical composition (% dry wt, w/w): cellulose 41.2%, hemicelluloses 34.4%, lignin 17.1%, ash 3.4%, extractives 0.5% and ethanol solubles 2.3%. Syringaldehyde was the predominant phenolic component, which comprised 65.6–68.5% of the total phenolic monomers in the oxidation mixtures. This basic result may explain the high degree of biodegradability of oil palm as this unit is more susceptible compared with guaiacyl containing wood lignin (Schwarze, 2007). Vanillin was the second major phenolic component. The presence of syringaldehyde and vanillin resulted from the degradation of non-condensed syringyl and guaiacyl units, respectively. The lower yields of alkaline nitrobenzene oxidation of these lignin fractions indicated a higher degree of condensation of the isolated lignins compared with the corresponding yields of hardwood lignins. The authors found that the lignin in oil palm trunk contained a high proportion of aryl ether-linked syringyl units. The presence of (i) a large proportion of non-condensed syringyl, (ii) a small amount of guaiacyl and (iii) fewer *p*-hydroxyphenyl units indicated that the fractions can

be considered as straw or grass type lignin. This is helpful as more information is available on these lignins compared with that for oil palm.

Wood Decay

General

Schwarze (2007) provided an excellent overview based on microscopic observation of wood decay and emphasizes that the terms for white and other rots can usefully be retained. Dry rot was not discussed. However, the rots caused by *Ganoderma adspersum*, *Ganoderma pfeiffera* and *Ganoderma* sp. on the other woods, may provide a model for *Ganoderma* decay of oil palm. Spatial arrangement of the woody tissue is relevant to decay as this is how fungi overcome host barriers. Angiosperm wood which contains guaiacyl-rich lignin is more resistant than syringyl-rich lignin. This is required to be taken into account for modifying oil palm lignin (i.e. it may be possible to increase guaiacyl units in oil palm lignin). However, specific data related to oil palm are limited.

Palm decay

There is no information linking the oil palm *Ganoderma* (recently referred to as *G. boninense*) directly with it being a white-rot fungus. For example, *G. boninense* has not been demonstrated to cause a typical white rot of oil palm – it simply has not been researched. Even the production of lignin-degrading enzymes has not been demonstrated from the fungus. Nevertheless, Rees (2005) states that *G. boninense* is 'a white-rot basidiomycete' and is the main threat to oil palm production in southeast Asia. Infection is postulated to occur by root contact but basidiospores may account for pathogen variation and infection of upper stems. Although why they could not cause basal rot remains unexplained. The production of lignin degrading enzymes by *G. boninense* has not been demonstrated except for in an unedited conference report (Ali et al., 2004) which also demonstrated that the fungus could reduce lignin in oil palm.

The most relevant paper on degradation of oil palm by *Ganoderma* is Adaskavej et al. (1991) which describes trunks of Canary Island date palm (*Phoenix canariensis*) being decayed by white- or brown-rot fungi. White-rot fungi caused significantly more weight loss (63%) than brown-rot fungi (32%). Of the white-rot fungi, *Ganoderma colossus* resulted in the greatest weight loss (81%), while *Scytinostroma galactinum* caused the least (36%). Chemical analyses indicated that both white and brown-rot fungi decreased starch, holocellulose and lignin. However, white-rot fungi removed greater amounts of lignin than the brown-rot fungi with three species, including *G. zonatum*, causing selective delignification (*G. zonatum* is related closely to the *Ganoderma* that rots oil palm). Scanning and transmission electron microscopy demonstrated that phloem and parenchyma cells were more susceptible to decay than xylem and fibre cells. In wood decayed by white-rot fungi, cell walls were eroded and middle

lamellae were degraded and selective delignification was observed in fibres adjacent to vascular tissue. Fundamental work of this nature requires to be undertaken for oil palm where *Ganoderma* is problematic. However, there is some information on rate of decay of oil palm and the rates of decay of the enzymatically digested oil palm in a study by Paterson et al. (2000).

Lignin Manipulation in Other Plant Systems

It is important for the advancement of transforming oil palm to consider what has been reported in other plant systems. Lignin configuration in transgenic and mutant plants was assessed by Anterola and Lewis (2002). The review deals with reducing lignin in plants as there are few data on increased lignin in modified crops. Distinct and sophisticated monolignol-forming metabolic networks are operative in various cell types, tissues and organs, and form the cell-specific guaiacyl (G) and guaiacyl-syringyl (G-S) enriched lignin biopolymers. In summary, depletion in monolignol supply reduced lignin contents and vascular integrity, with a concomitant shift towards (upstream) metabolite build-up and/or shunting.

Furthermore, lignification of plant cells at sites of infection or lesions is a defence response of plants to decrease pathogen spread. Peroxidase is required for the final polymerization of phenolic derivatives into lignin and may also be involved in suberization or wound healing. For example, a decrease in polyphenolic compounds, such as lignin, in potato tubers by redirection of tryptophan in transgenic plants through expression of tryptophan decarboxylase, rendered tissues more susceptible to *Phytophthora infestans*, illustrating the role of phenolic compounds in defence. On the other hand, reduction of phenylpropanoid metabolism via inhibition of phenylalanine ammonia-lyase activity in transgenic tobacco rendered tissues more susceptible to *Cercospora nicotianae*. Overexpression of a cucumber peroxidase gene in transgenic potato did not increase resistance of tissues to infection by *Fusarium* or *Phytophthora*, and lignin levels were not significantly affected in spite of elevated peroxidase expression. Obviously, the unaffected lignin concentrations may be the reason that resistance was not increased. On the other hand, overexpression of a tobacco anionic peroxidase gene in tomato did enhance lignin levels although resistance to fungal pathogens was not enhanced. Lignin levels were also significantly higher following expression of the H₂O₂-generating enzyme glucose oxidase (GOD) in transgenic potato (Wu et al., 1997) and by expression of the hormone indoleacetic acid (IAA) in transgenic tobacco. It is remarkable that tolerance to several fungal pathogens was enhanced. In summary, lignin protects plants from attack by pathogens (Punja, 2001).

The increase in lignin content in *Bt* corn may be beneficial as it can provide greater resistance to attack, hence reducing susceptibility to fungi (Saxena and Stotzky, 2001). Leaves of transgenic tomato plants overexpressing a peroxidase contained more lignin

than the isogenic untransformed plants which was unpredictable. A transgenic potato line expressing the GOD enzyme had a significant increase in resistance to *Phytophthora infestans* and also had a higher stem and root lignin concentration. RC332 tomatoes plants had the *Gox* gene encoding GOD enzyme. Also, the transgenic plants contained more insoluble lignin than the control plants (Marinari et al., 2004).

A few reports exist concerning *Bt* corn, which have focused on lignin. *Bt* corn had significantly higher lignin content than near-isogenic non-*Bt* corn and the lignin content of other *Bt* plants tested were 10–66% higher. As anticipated in Paterson (2007) for oil palm, changes in the amount, composition or conformation of lignin may alter the amount of protection offered to polysaccharides, proteins, etc., making them more susceptible to biodegradation and influencing the rates of decomposition (Flores et al., 2005). Furthermore, active oxygen species have been postulated to perform multiple functions in plant defence, but the exact role in plant resistance to diseases is not fully understood. The authors demonstrated H₂O₂-mediated disease resistance in transgenic potato plants expressing a foreign gene encoding GOD. Further evidence suggests that the H₂O₂-mediated disease resistance in potato was effective against a broad range of plant pathogens. In addition, an increased accumulation of several isoforms of extracellular peroxidase was observed, which was accompanied by a significant increase in the lignin content of stem and root tissues, and this contributes to the H₂O₂-mediated disease resistance in transgenic plants (Fig. 1) (Wu et al., 1997).

Transformation of crops, including maize with the *cry1Ab* gene from *Bt* to combat lepidopteran pests, resulted in pleiotropic effects regarding lignin biosynthesis. The stems of the transgenic lines had higher concentrations of total lignin than the respective isogenic

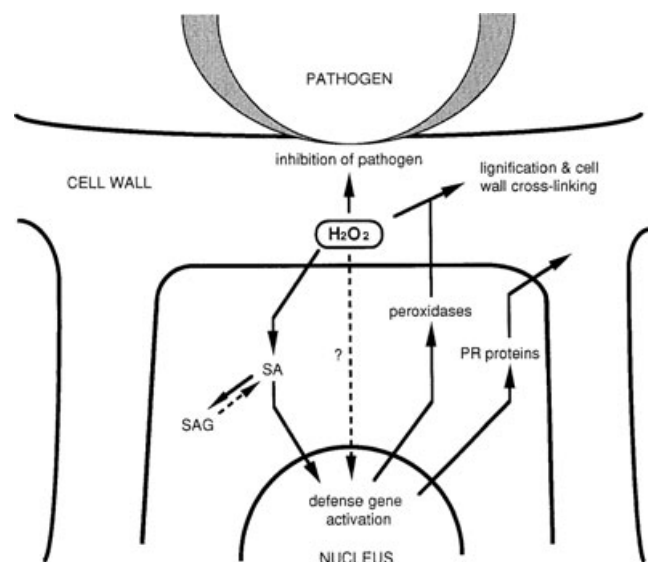


Fig. 1 Host defence activation by H₂O₂ produced extracellularly in transgenic plants. The increase and induction of peroxidases contribute to lignification and cell wall cross-linkages. This leads to enhanced disease resistance in plants (see Wu et al., 1997)

lines. There were significant modifications in the ratio of *p*-hydroxyphenyl/guaiacyl/syringyl molecular marker units of stem lignin, and the guaiacyl units accounted chiefly for the higher total lignin contents. Stems of *Bt* plants had higher lignin content from 33% to 97% than stems from the non-*Bt* lines (Saxena and Stotzky, 2001). The increase in total lignin can be explained by the transgene being inserted unintentionally into genes that control lignin biosynthesis. It is assumed conventionally that the G type lignin is more resistant to biological breakdown (e.g. oxidation and demethylation) than the S type. The G type have a 5- aromatic position available for strong C–C bonds which makes them resistant and this position is blocked by methoxy groups in the case of the S type (Poerschmann et al., 2005). Obviously, the *Bt*-modified oil palm discussed in Hashim et al. (2002) requires to be assessed for lignin content and *Ganoderma* resistance.

In addition, Cu-treated peanut seedlings demonstrated a significant inhibition in peanut root growth and a decrease in endogenous IAA content. The increase in the activity of anionic peroxidase (POD) isozyme P3.5 was correlated with the rise in lignin content in Cu-treated roots. The authors suggested that the increase in anionic POD isozyme P3.5 induced by Cu might be responsible for lignin synthesis in peanut roots (Li et al., 2001). Again, these procedures await experimentation with oil palm.

Genetic modifications of plants can alter properties in ways that are not completely predictable. Modification of hemicelluloses is proposed as an alternative strategy to direct lignin manipulation as a target for testing the effects on (i) plant growth and (ii) cellulose content and extractability. Tobacco has been the model woody plant of choice for much of the work on modifications of lignin and was chosen for the study of the comparative effects of modification of xylan by Bindschedler et al. (2007). The authors suggest that the level of xylan relative to lignin is an important factor in delignification. Although progress has been made in identifying the genes involved in the synthesis of cell wall components such as cellulose and lignin, identifying those involved in the synthesis and modification of other components is less complete. What is the situation with oil palm where there are fewer data?

Oil Palm Manipulation

Resistant varieties

Resistant varieties may hold the greatest hope for future BSR control of oil palm in southeast Asia due

to the apparent unacceptability of genetically modified organisms in Europe. Field observations in North Sumatra revealed *E. guineensis* (Deli type) originating from Malaysia and Indonesia was more susceptible than African material, and other trials have revealed differences in susceptibility. These indicate possible genetic resistance within host populations and perhaps differences in pathogenicity among *G. boninense* isolates (Rees et al., 2007). As mentioned above, Idris et al. (2002) and Durand-Gasselin et al. (2005) observed high susceptibility of Deli material and low susceptibility of African material. Durand-Gasselin et al. (2005) suggested that there was insufficient evidence to support the hypothesis of a difference in susceptibility between *E. guineensis* and *E. oleifera*.

Oil palm breeding has been undertaken frequently with the subsidiary objective to give tolerance to disease (the primary aim is to maximize oil and kernel yield). In the present context, the genetic variation for lignin concentration within oil palm populations needs to be determined. Oil palm is monoecious and is naturally cross-pollinated. The most important monoecious crop is maize and may be a model for oil palm breeding.

Plant breeders work with variation. Any changes in vegetative characters such as lignin concentration result from alterations in genes. Furthermore, mapping and linkage studies may allow selection of high lignin at the nursery stage. Two relevant characters are (i) resistance to wind and (ii) height. Conceivably, these may relate to the chemical constituents of the oil palm trunk and would be worth investigating. Variation in disease resistance in oil palm has been demonstrated and breeding has been most successful with *Fusarium* wilt, although an investigation for molecular makers to wilt was unsuccessful (Corley and Tinker, 2003).

Transformation of oil palm

Oil palm is being researched intensively by the Malaysian Palm Oil Board as an 'anchor institution' (Hashim et al., 2002) (Table 1). Conventional oil palm breeding is inefficient and time-consuming. However, current breeding programmes combine conventional and modern biotechnology approaches. Although many studies have been carried out on genetic manipulation of oil palm, publication of the results is limited. This may be from commercial interests and/or the long oil palm generation cycle. Intellectual property rights are important in this respect.

Obviously, the position is advancing and the lignin content of the *Bt* oil palm needs to be investigated, as

Table 1
Transformed oil palms which were in development (Hashim et al., 2002)

Property	System	Stage
Oil quality improvement	Stearoyl-acyl carrier protein desaturase and keto-acyl synthase	Transformation system developed
Herbicide tolerance, insect resistance	Transformation system developed, CPT1a; <i>Bt</i> gene	4½-year-old transgenic plants obtained in planthouse; ready for screening
<i>Ganoderma</i> resistance	Chitinase	3-year-old transgenic plants obtained in planthouse; undergoing screening

some other *Bt* plants have higher lignin (see the Lignin Manipulation in Other Plant section above). Transgenic palm oil plants with (i) resistance to hygromycin, (ii) glucuronidase activity and (iii) herbicide resistance have been investigated. Molecular analysis and physical screening confirmed the expression of the transgenes in the regenerated plants, obtained by both *Agrobacterium*-mediated and direct DNA transfers through particle delivery systems. Recently, significant progress has been reported with *Agrobacterium*-mediated transformation of chitinase into oil palm, and transgenic and chimeric oil palm plants carrying the cowpea trypsin inhibitor gene were found to be resistant to bagworm larvae. Genes of the fatty acid biosynthetic pathway of the oil palm have been cloned, characterized and used for gene construction. Vectors have been constructed with inserts of stearyl-acyl carrier protein desaturase and keto-acyl synthase 1 genes for oil palm transformation, and the isolation and characterization of cDNA clones encoding for oil palm thioesterase have been reported. However, there is limited availability of construct elements (e.g. genes, promoters, enhancers, terminators and other DNA sequences) and of scientific literature on oil palm. Furthermore, the creation of clonal populations of oil palm with high lignin content may be possible. Clones can be produced by tissue culture where 'explants' are grown on nutrient solutions. However, these techniques are difficult, slow and not well understood.

The approaches that have been taken can be grouped into five categories for oil palm:

The expression of gene products

- (1) that can potentially enhance the structural defences in the plant. These include elevated levels of peroxidase and lignin;
- (2) that are directly toxic to pathogens or reduce growth. Such work on chitinases has been undertaken with oil palm to control *Ganoderma*;
- (3) that destroy or neutralize a component of the arsenal of the pathogen, such as polygalacturonase, oxalic acid and lipase. *Ganoderma* lignin degrading enzymes may be negatively affected by developing an enzyme inhibitor (Paterson, 2008) producing gene;
- (4) that release signals regulating plant defences;
- (5) concerning resistance involved in the hypersensitive response and in interactions with avirulence factors (Punja, 2001).

In addition to application of the Biolistics method, studies on transformation mediated by *Agrobacterium* and utilization of the green fluorescent protein gene as a selectable marker gene have been initiated.

Biolistics

Biolistic methodology was initially chosen as the method for oil palm transformation because of the success with other monocotyledons. Optimization of physical and biological parameters, including testing of promoters and selective agents, was carried out as

a prerequisite for stable transformation. This has resulted in the successful transfer of reporter genes into oil palm and the regeneration of transgenic oil palm, thus making it possible to improve the oil palm through genetic engineering. The problem lies in assessing the functionality of transgenes in putative transformants and this could not be demonstrated clearly. However, several expression vectors carrying the *CryIA(b)* gene were constructed and transformed into oil palm which was clearly demonstrated by analysis on the transgenes functionality in the host. A rapid technique is provided to screen for putative transformants that proved very difficult in gene manipulation of monocots, especially in slow growing oil palm (Lee et al., 2006). In oil palm, the condition for delivering DNA into embryonic calli has been based on transient 3-glucuronidase (*GUS*) gene expression. Five constitutive promoters (*Emu*, *ubiquitin*, *actin*, *cauliflower mosaic virus* and *Adh1*) were evaluated and significant effects on transient *GUS* gene expression were demonstrated by each of the different promoters tested. Kanamycin, geneticin, neomycin and hygromycin antibiotics and the herbicide Basta4 were tested as selection agents to inhibit growth of untransformed cells; hygromycin and Basta4 were found to be most suitable. Furthermore, the efficiency of *GUS* gene expression in embryogenic callus and young leaflets of mature and seedling palm after microprojectile bombardment with five constructs was evaluated to identify the most suitable promoter(s) to use in transformation attempts in oil palm in the first such report. Two promoters, *UbiI* or *Emu*, were suggested to be the optimal promoters to develop a genetic transformation system for oil palm (Chowdhury et al., 1997).

Agrobacterium-mediated transformation

Progress of this method on oil palm remains limited. However, immature embryos (IEs) were transformed with pCAMBIA 1301 using the sonication-assisted *Agrobacterium*-mediated transformation method. This technique enhances the transient transformation efficiency, 30 ± 45% more than the non-sonicated technique. However, the efficiency is still low and inconsistent. Particular emphasis was placed by Lee et al. (2006) on developing techniques for genes transfer into oil palm using direct gene and *Agrobacterium*-mediated approaches.

Green fluorescent protein

In an interesting experiment, six constructs carrying the green fluorescent protein gene driven by different promoters were used to bombard oil palm embryogenic calli. Generally, the number of 'spots' and their brightness declined drastically after 3 days of bombardment and were invisible after 2 weeks. However, embryogenic callus bombarded with a 35 S-SGFP-TYG construct, showed the highest number of spots and a few of the spots retained expression for more than 5 months which was encouraging (Parveez et al., 2000).

Immature embryos

Early results from several field trials on clonal palms have shown good yield improvement. Complete plants have been successfully regenerated from various explants of oil palm. However, the frequencies for complete plant regeneration from some explants are still low. The introduction of foreign genes into oil palm has been limited due to the lack of an efficient, reliable and rapid regeneration system. But the ability to regenerate complete plants from all the above explants has made oil palm amenable to genetic manipulation for the incorporation of foreign gene(s). Not all explants were suitable for genetic manipulation studies.

Abdullah et al. (2005) investigated the potential of IEs as a useful tool for oil palm genetic transformation studies. It was evident that IEs were amenable to direct and *Agrobacterium*-mediated gene transfer. Due to the abundant supply of IE, optimization of biolistic and *Agrobacterium*-mediated gene transfer into IEs were carried out 'without undue problems'. IEs were also more responsive to *in vitro* manipulations compared with other explants such as leaf and root tissues. Rapid *in vitro* response to callusing and embryogenesis, or rapid and highly efficient direct germination resulted in a shorter culture period. This will presumably minimize the production of abnormal clonal palms, which has been associated with chromosomal aberration due to prolonged time in culture. In addition, IEs allow rapid and direct introduction of elite genes into breeding programs and in biclonal seed production.

It is envisaged that commercial planting of transgenic palms will not occur any earlier than the year 2020 due to the long life cycle of the palm and the time taken to regenerate plants in tissue culture. Oil palm has a long generation time (7 ± 10 years) and present planting materials are derived from a narrow gene pool, which restricts the introduction of new traits. On the other hand, only 4–5 years are required to produce transgenic plantlets carrying a new trait. Genetic engineering could save $80 \pm 90\%$ of the time required for introducing a new gene trait into oil palm taking into account the requirement for back-crossing in conventional breeding. More importantly, whereas crossing two distinct parents *via* oil palm seed production will produce a population of related palms in the plantation with a range of resistances, genetic engineering would produce a uniform population with total or partial resistance. This implies that a control screening process *via* seed production of oil palm needs ideally to be compared with the altered lignin oil palm.

Conclusions

The role of *Ganoderma* on oil palm as the cause of a white rot disease has been neglected. This type of attack involves the degradation of lignin as the defining mode. Much more knowledge is required on the biochemical structural of the macromolecules in oil palm, and how they vary within and between species. In addition, the ligninolytic enzymes in the *Ganoderma*

involved in oil palm disease require being determined. An obvious approach for control is to develop oil palm with altered lignin so that it is resistant to attack. The technology exists in other plants and other forms of transformation have been undertaken with oil palm. Oil palm lags somewhat behind the other crops. However, it is possible that an oil palm can be produced with resistant lignin to *Ganoderma* disease. The lignin content of *Bt* oil palm needs to be determined. Genetic engineering may not be the only procedure which can be employed; the natural lignins in the different species and variation within species could be assessed. The length of time for introducing improved plants into the plantation can be decreased. Finally, the intellectual property rights of those producing modified oil palm need to be determined well in advance of producing modified oil palm.

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