# Diversity of Plants – A Molecular Approach

*Editor*

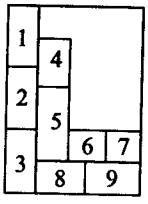
**S John Britto SJ**

2009 - First Edition


*Price* Rs.350; £ 60; $ 80.

**ISBN** : 978-81-908503-0-8

**Cover Illustrations**

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*Published by*

The Rapinat Herbarium *and*
Centre for Molecular Systematics
St. Joseph's College (*Autonomous*)
Tiruchirappalli 620 002

# Strategic Application of Biotechnology for Improvement, Management and Exploitation of Oil Palm in India.

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## Abstract

Oil palm is the highest edible oil producing crop which has the potential of meeting the oil demand of our country. In India, oil palm was introduced as irrigated crop and it has been doing extremely well. Biotechnological approaches have a major role for the sustainable development of the crop. However, it is important to set the priority areas of biotechnology for the improvement, management and also utilization of this palm. Areas under crop improvement like clonal propagation of elite palms and marker assisted breeding needs major emphasis. Work on development of transgenic oil palm with important agronomic traits can be initiated. The molecular and biotechnological tool for disease management is another important area, which is discussed in the article. Finally, the non conventional use of the plant products, metabolites and genes as part of bio-prospecting has been elaborated. In total, this paper discusses the application of Biotechnology in priority areas of oil palm research under Indian context.

## Introduction

Oil palm is a crop which is the most efficient energy converter and can produce maximum quantity of edible oil per unit area. An unbelievable figure is at sight which is 18 tons of oil per hectare (Rethinam, 2008). An all round approach to efficient utilization of this natural bio-engine can lead to a country to high economic status; Malaysia and Indonesia are the examples. We have introduced this crop in India towards the end of eighteenth century as a botanical collection at National Botanic Garden, Calcutta (Wahid Basir *et al.*, 2005). But as a crop it has a history of about 40-45 years which was started in Thodupuzha, Kerala. Initial introduction at Kerala was under rainfed condition and in the areas of tropical rain forest. Utilizing the full potential of the crop and expanding to the farmers' field was not much due to the constraints of climatic and geographical condition and high value land. Around two decades back, efforts were taken by a few visionary agricultural scientists and policy makers to bring oil palm cultivation in India under irrigated conditions (Chadha, 2006). Oil palm is usually cultivated under rainfed condition with maximum and minimum temperature reported to be 36°C and 18°C and the best mean temperature range seems to be 24-28°C (Corley and Tainker, 2003). Against the several

odds, the crops is established as irrigated crop in different states of India where the maximum temperature touches 49°C (Andhra Pradesh) and goes down below 11°C (Mizoram). However, the full potential of the crop is not yet been realized.

Biotechnological intervention has an important role in agriculture and hence in oil palm too. The potentiality of the tool is enormous and left to the logical thinking of the scientists, which can be effectively put to application. Through biotechnology several things can be achieved but they may not be the priority or some may not be required. In this article we elaborate the different areas of priority to be taken up using the biotechnological tools for this crop under the Indian context.

**_In vitro_ propagation**

Unlike other conventional field crops, oil palm has no released variety so far, mainly because it is a cross pollinated crop and having breeding cycle of more than 10 years (Low *et al.*, 2008). Developing a pure line may take several years. The palms, which are grown commercially in the farmers' field, are different from each other. Hence, the performance of the palm also varies from each other. No simple method of vegetative propagation is available for oil palm and thus, propagating a superior palm is only possible through *in vitro* regeneration. Therefore, tissue culture is the most important aspect of biotechnology, which is to be employed for the crop improvement of oil palm. In India, so far there is no regeneration protocol available from mature palm, though the same is available in other countries. Many agencies are commercially producing clonal plants and selling at a premium price. In India, efforts have been started at the National Research Centre for Oil Palm and results are expected within a couple of years.

**Strategies for _in vitro_ regeneration**

A few important aspects under tissue culture needs to be discussed for understanding the real issues. Mass multiplication of *tenera*, which is a commercial hybrid all over the world, is derived by crossing *dura* X *pisifera* (D X P). *Dura* is a thick shell fruit type having very less economic part of the fruit (mesocarp and kernel), where as *pisifera* is shell less type but palms rarely produce bunches. Hence, the *tenera*, which is a natural hybrid of *dura* (female) X *pisifera* (male), produces thin shell fruits having higher mesocarp and kernel and thereby higher amount of oil. *Dura*, *tenera* and *pisifera* segregate in 1:2:1, when a *tenera* is crossed with *tenera* (selfing or *inter-se*-mating). Presently systematic crossing of high yielding *dura* with *pisifera* (derived from a superior pedigree) are carried out and the performance of the progeny is evaluated in the field and

if the average yield of the progeny is found satisfactory, the parental combinations are used for seed production. However, by the time progeny testing results are confirmed, the age of the palm reaches 15-20 years and their utilization period gets limited. A palm can be accessed for seed production for a maximum period of 40-45 years, after which height becomes a limiting factor. Thereafter there is no chance of producing the same hybrid and the combination is lost for ever. Hence, primarily a superior *tenera* hybrid should be mass multiplied through tissue culture. But the problem of somaclonal variation always haunts. Even a low percentage of abnormalities can cause considerable loss in a plantation. Some percentage of somaclonal variation is common, because requirement of growth regulators is relatively more in case of oil palm tissue culture. Alternative proposition is the mass multiplication of the parents. Once a superior D X P combination is identified, the same D x P can be multiplied through tissue culture. In this case the palm could be tested for their performance as well as abnormalities in the field and the true to type palms could be used for seed production. This is called bi-clonal seed production. When a single palm can produce $12 \times 1500 = 18000$ seed per year, the number can be increased to as many folds as the number of true to type clonal plams is produced. Since number of *pisifera* required is much less than of *dura*, mass-multiplication of elite *dura* can suffice and seed production can be done by crossing clonal *dura* with normal *pisifera*, which is called monoclonal seed production.

Another important use of tissue culture is for regeneration of transformed cell for production of transgenic. The desired genes for transformation are discussed later. Tissue culture protocols needs to be there for cryopreservation also. The research on cryopreservation needs special attention and it has to be integrated with a tissue culture laboratory for revival of the cryopreserved materials time to time especially embryos and somatic embryos.

**Molecular marker**

Use of molecular markers is an important area of crop improvement, which has got more prominence due to its accuracy and the capacity to identify the plants with desirable traits much before it is expressed. However the application of markers, mainly DNA markers are for different purposes. Different marker systems, which are commonly used are Restriction Fragment Length Polymorphism (RFLP), Randomly Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP) or Simple Sequence Repeat (SSR) or microsatellite. A convenient marker system or a combination of them is used for different purposes.

**Genetic diversity study using DNA markers**

As in the case of any other crop, assessment of genetic relatedness of different palms/varieties/populations etc. is extremely important before taking up breeding programme. Using different marker mentioned above, the DNA of different individuals are tested for their similarity or dissimilarity at a particular locus. In principle their differences in restriction site (RFLP and AFLP), in the primer binding (RAPD and AFLP) site or differences in the number of repeat sequences (SSR) exhibit in terms of number of similar or dissimilar bands, which is scored as binary code 1 and 0 or absent or present and they are analyzed statistically to calculate the differences. In case of oil palm, this is carried out to determine the genetic diversity and the data is very important for taking up crossing programme as the genetically diverse material is the source of heterosis. In these studies, the marker exhibiting similar or dissimilar bands among the individuals are equally important because the information of locus which is similar in DNA sequences is equally important as much it is for the locus showing the differences in DNA sequence. True genetic diversity can be calculated by combining the data. While analyzing the genetic diversity, more the number of loci is covered, more the result will be reliable. Genetic diversity and relationship between breeding populations of oil palm by using DNA markers are reported from the workers from other countries (Shah *et al*, 1994; Mayes *et al*., 2000; Moretzsohn *et al*, 2002). In India, five of the *dura* germplasm accessions available, namely ASD1 (98C - 254 D) and ASD2 (98C- 208 D) from ASD Costa Rica, and PLD1 (GDD3), PLD2 (240D x 281 D) and PLD3 (80D X 281 D) from Palode, Kerala, India were analysed for their genetic diversity using RAPD (Mandal *et al*., 2004). The primers used were also evaluated for their usefulness to be employed for palm identification (Table 1). This was the first attempt of molecular characterization of some of the oil palm germplasm accessions available in India. This study revealed a high degree of DNA polymorphism among the different palms and also among the different accessions (under study) of oil palm germplasm. Subsequently five exotic accessions (One each from, Guinea Bissau, Zambia, Tanzania, Cameroon and ASD Costa Rica) of oil palm have been analyzed for the biochemical and molecular characterization along with one indigenous accession from Palode. This study by RAPD analysis showed six different groups, each consisting of palms from same accession, although no two palms from any accession were completely similar (Fig.1). Palms from Guinea Bissau accession were highly homogenous in comparison to other groups and the same accession was genetically more distant from others. Cameroon and ASD Costa Rica accessions were found closer to each other. (Mandal and Susmita, 2006).

***Table 1*: Grouping of 33 primers used for RAPD analysis of five accessions of oil palm as per their usefulness for palm identification.**

| | Most Useful → Least Useful | | | | | |
|---|---|---|---|---|---|---|
| | **Group-I** | **Group-II** | **Group-III** | **Group-IV** | **Group-V** | **Group-VI** |
| **Primers** | OPP-05 | OPM-17 | OPN-14 | OPN-10 | OPM-12 | OPM-07 |
| | OPM-04 | OPN-20 | OPN-02 | OPO-09 | OPO-10 | OPN-11 |
| | OPN-05 | OPN-09 | OPP-06 | OPN-06 | OPN-04 | OPN-13 |
| | | | OPN-03 | OPM-14 | OPO-08 | OPN-16 |
| | | | OPN-15 | OPM-15 | OPN-08 | OPN-17 |
| | | | OPN-12 | | OPM-11 | OPN-19 |
| | | | OPM-18 | | | OPP-04 |
| | | | OPO-11 | | | |
| **Total No. of Bands** | 24 | 28 | 51 | 25 | 22 | 17 |
| **Polymorphic band (%)** | 91.66 | 64.29 | 82.35 | 76.00 | 45.45 | 0.00 |
| **Mean EMR** | 7.33 | 6.00 | 5.25 | 3.8 | 1.67 | 0.00 |
| **Mean $H_{av(p)}$** | 0.69 | 0.79 | 0.62 | 0.58 | 0.30 | 0.00 |
| **MI** | 5.06 | 4.76 | 3.26 | 2.21 | 0.51 | 0.00 |

EMR: Effective multiplex ratio; $H_{av(p)}$: Average heterozygocity (for polymorphic band); MI: Marker Index

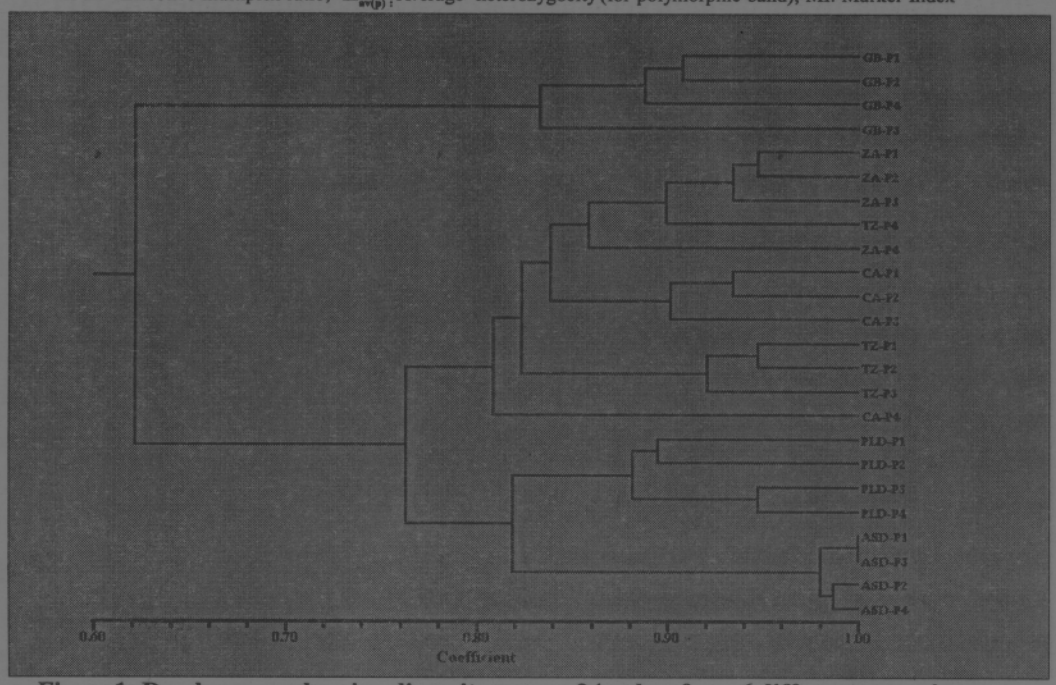



***Figure* 1: Dendrogram showing diversity among 24 palms from 6 different accessions.**

Other than routine analysis of genetic diversity of oil palm germplasm accessions, it has some other important applications in breeding too. As it is discussed earlier that a hybrid combination is selected as superior after the evaluation of its progeny, the parents can be exploited for a limited period after the combination is confirmed to be superior. In conventional breeding, the *dura* parents are selfed to produce the progeny and in the next generation, better performing *dura*s are selected as mother for producing seed, though the genetic configuration cannot be the same as the previous (*dura*) parents. Genetic variability study using DNA markers can detect the progeny palms which are closest to their parents and they can be employed for breeding and seed production and the strategy can be adopted for routine use.

Similarly, the genetic fidelity of the clonal plants can be tested by using DNA markers. Though it is reported that the abnormalities (somaclonal variations) are not detected by DNA fingerprinting (Corley and Tinker, 2003), might be because most of the cases the differences are not in the DNA as such but due to its expression.

DNA fingerprinting for identification of elite material and to resolve IPR issues are important even in case of oil palm, though practical use of the same is not being reported so far.

For the last two applications mentioned above i.e. the genetic fidelity study of clonal palms and fingerprinting for identification of individual palms and their progeny need markers which are polymorphic in nature unlike in case of genetic diversity study, which requires both polymorphic as well as monomorphic markers.

**Marker Assisted Selection of desirable traits**

In case of marker assisted selection (MAS), a particular character or trait is identified with a DNA marker. Finding markers linked to useful traits is more difficult, in that detailed statistical analysis of the segregating population for the trait of interest is required, and a large numbers of markers may have to be tested before linkages are found. DNA marker technology has been used by Jack *et al.* (1998) for tagging qualitative (simply-inherited gene) and quantitative genes. They presented a RFLP linkage map of oil palm and tagged two genes, namely the shell thickness (Sh) and virescence (Vir). Different linkage groups were established for different quantitative traits.

As discussed earlier, oil palm being a plantation crop and very long breeding cycle, it requires considerable time for selection and developing variety. Marker assisted selection would definitely help in reducing the time for breeding programme at the same

time it would be more accurate selection than that of the conventional method. However, the major issue concerned to the MAS in oil palm is the development of mapping population. Several accessions are available in India, which requires selection of the palms for desirable traits. Selection of palms for different traits has been started and it would take definitely a few years to select the palms with different contrasting characters for a desirable trait. Crossing can be made only after the selection, however from the time of making a cross to production of sprouts itself takes around one year. Thereafter raising the segregating population and recording the phenotype for the desirable traits would take further 10-12 years. However, in case of a shell thickness marker, a segregating population (F2 population) is available in all the seed gardens, which could be used as mapping population.

Similarly the contrasting character for fruit color (*virescens* and *nigrescens*) can be crossed immediately for developing a mapping population, importance of which is discussed later. Other than these two traits, though many other important traits are to be considered for MAS, developing a marker for these characters would take at least a decade if we start the programme now.

**Bulk segregation analysis (BSA)**

The other approach of MAS without mapping population can be bulk segregation analysis. Quantitative trait locus analysis is usually associated with a mapping population of plants, each of which has to be (a) genotyped with all the markers selected to cover the genome, and (b) phenotyped for the traits of interest. Genotyping a large mapping population is tedious and relatively costly in terms of consumables. By grouping plants according to either high or low expression of a particular trait and extracting DNA from these two bulks, the process of genotyping the plants is reduced to only two DNA samples to be analyzed instead of having to analyze DNA separately from each plant. Two variants of the BSA technique are possible depending on whether these plants are derived from a cross between two parental lines or from a population of plants with diverse genetic back- grounds (e.g. variety mixes or composite populations). For BSA of the trait of interest, parental lines are chosen that differ in their expression and crossed, and, as with QTL analysis, $F_1$, doubled haploid or recombinant inbred populations are generated which will segregate for the trait. Although Michelmore *et al.* (1991) was successful in identifying markers linked to a resistance gene in lettuce using $F_2$ plants, for traits controlled quantitatively or by a single recessive gene, doubled haploids or recombinant inbreeds will increase the probability of locating markers linked to gene(s) controlling the trait. The

population is then phenotyped to identify individual plants or lines having high or low expression of the trait. Two DNA bulks are prepared, one from the 'high' individuals and the other from the 'low' individuals (for example 10 'high' and 10 'low' from a population of 200 individuals), and analysed for allele frequency with molecular markers. With dominant markers such as RAPDs, this would apply whether the individuals came from a single segregating population or from pools of genetically diverse individuals (Quarrie *et al.*, 1999).

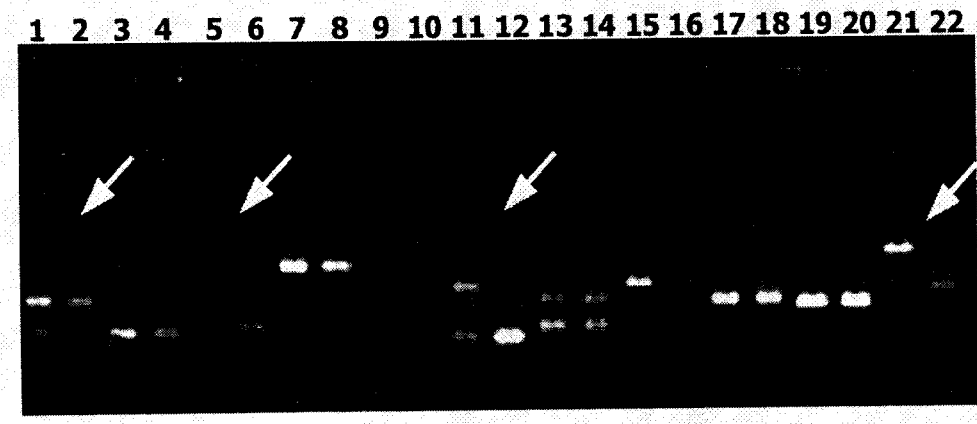
The second variant of BSA applies when using pools of genetically diverse individuals, such as variety mixtures or composite populations of outbreeding species, and differs from the first only in the number of alleles likely to be present at any marker locus.

In case of oil palm BSA from a F2 population of T X T cross can be effectively used for the identification of shell thickness marker, which is known to be controlled by single gene. This seems though easy, so far a marker for the practical purpose has not been derived. BSA was applied to identifying AFLP markers linked to the *Sh* (shell thickness) gene. One AFLP marker of the shell allele coding for a thick shell was identified (Billotte *et al.*, 2006). Earlier Mayes *et al.* (1997) reported RFLP marker for the shell thickness. However, the markers are not been used as they are not robust. At National Research Centre for Oil Palm, preliminary attempts have been taken to screen PCR primers and selected the probable primers linked to the shell thickness (Mandal and Pillai, 2005). 'Bulk segregation' approach was adopted for the study and DNA isolation was carried out from 25 *dura* and 25 *pisifera* palms. Two pooled DNA samples were subjected to RAPD analysis with 50 random primers using the standard RAPD protocol. The amplified samples were electrophoresed and documented. Both the pooled samples showed same banding pattern with all the primers, except 10 primers showing differences (Fig. 2). Still the hunt for a reliable marker is on and the work is continuing at NRCOP also.

The need for the shell thickness marker for early identification of the palms is beyond doubt. This is because the farmers are provided with the hybrids i.e. the *tenera* seedlings, which are sometimes contaminated with *dura* population. This causes a perennial loss of oil yield since *dura* fruits contain lesser oil. Secondly, the maintenance of *tenera* X *tenera* population as seed garden with unwanted palms is a necessary evil at present, since only *dura* and *pisifera* palms are required for seed garden. Moreover for the breeding programme and for germplasm maintenance, planting right type of plant variety is extremely important, which so far could not happen due to lack of marker and varietal identification.

Similar to varietal identification, fruit type identification of *virescens* (*green* immature fruit) and *nigresens* (purple immature fruit) at early stage is very important to plant the right type of palm. *Virescens* character is important because it changes fruit colour distinctly from green to orange and the maturity can be identified easily. Moreover it is thought to be energy efficient due to presence of chlorophyll in the fruits during the maturation. Other traits of importance for MAS are higher oleic acid content, dwarfness and drought tolerance. Though the application MAS with these traits may require more than a decade, the work should be started immediately, keeping in view the importance of the crop.

***Figure 2*: RAPD pattern of dura and pisifera pooled DNA with different random primers. Arrow mark indicating the difference in pattern between the two pools. Lane: Odd lanes are dura pooled DNA; Even lanes are *pisifera* pooled DNA. Lane 1&2, 3&4, 5&6 etc. are amplified with separate random primers**




## Genetic manipulation and transgenic oil palm

Elsewhere the transgenic oil palms are there in the field for their evaluation Ruslan Abdullah (2005), and commercial scale transgenic oil palm may be in the market within a few years. To start with, the transformation studies on oil palm should be initiated. The important aspect is the trait/ gene to be incorporated/ manipulated. Target gene will often be the gene coding for new enzymes, not already present to give the plant a function that it did not have, but two other sorts of changes are also possible. Gene activity can be increased by inserting additional copies of the gene, or activity can be eliminated by the antisense method. This involves the insertion of a synthetic gene with a DNA sequence that complements the target gene, and so cancels out its activity. In every case target gene must be combined with suitable promoters to regulate when and in which tissue they are active (Corley and Tinker, 2003).

Modifying a specific enzyme, high unsaturation is required for edible purpose. Hence the oleic acid content can be increased in the oil by increasing the activity of ß-Ketoacyl-ACP Synthase II (KAS II) or using antisense to reduce the activity Palmitoyl-ACP Thioesterase (PATE) or both (Soh *et al.*, 1994). Other possibilities are increasing the stearic content by reducing the activity of δ–9 desaturase; increasing the palimitic acid content by reducing the KAS-II activity, decreasing saturated fatty acids content by increasing δ-9 desaturase activity. (Parveez *et al.*, 1994). When changing the quality of oil is the objective, mesocarp specific promoter is very important. Shah and Cha (2000) identified mesocarp specific promoter from *E. oleifera*, which was not found in *E. guineensis*. However, it is the other eminent workers on oil palm considered that transformation may play a greater role in further yield increases and cost reduction than in the production of specialty oil (Corley and Tinker, 2003).

A decade of transgenic research had been reviewed by Ruslan Abdullah (2005). He reported that the first batches of transgenic plants produced were engineered to harbour the *cowpea trypsin inhibitor* (CpTI) gene and the plants are now more than 8 years old. The stability of transgene integration was continuously monitored throughout the plants life span using molecular techniques. Continuous bioassays on the 8 year-old CpTI plants showed increased tolerance to bagworm larvae (*Metisa plana* Walker) as compared to non-transformed controls. Using the same strategy but with different gene(s), work are in progress to address basal stem rot caused by *Ganoderma boninense* in oil palm. Recently, several genes involved in biosynthesis of fatty acids and bioplastics were successfully transferred into target tissues and regenerated into complete plants. Preliminary assessment indicates successful gene transfer. Functional expressions of genes leading to changes in fatty acid profiles and the production of polyhydroxybutyrate (PHB) have also been demonstrated. Whilst the golden crop is being transformed with other useful genes expressing novel traits, the techniques of gene transfer for oil palm is continuously improved with emphasis given on their efficiency, role of different promoters, enhancers, transgene stability and inheritance, gene targeting and also novel gene transfer techniques. In Indian context the most important traits to be considered for transgenic would be drought tolerance, which is not considered by any of the workers.

**Molecular Diagnostics**

This aspect is gaining importance mainly for the management of fatal diseases. Early identification or diagnosis of the diseases and causal organism can save the palms, which otherwise would have been detected at a stage, when the palms are at a terminal stage and not curable. With the advent of molecular biology tools, molecular diagnostic

of diseases has become a common practice in case of most of the important diseases of crop plants. It gives certainty of diagnosis, and detection of diseases at an early stage is essential for taking the proper remedial measures at the appropriate time. Yet another drawback is that the diseases can easily spread at this advanced stage and hence the chance of an outbreak exists. Hence, molecular diagnosis is a part of the integrated disease management system in agriculture. It is very important in case of oil palm, whose economic life is approximately 35 years, and a lot of investment goes into the management of palms and their diseases.

Though oil palm is affected by only a few diseases, two of the important diseases of oil palm, whose etiology is already reported are the basal stem rot (BSR) disease caused by *Ganoderma sp.,* and the spear rot disease (SRD) caused by Phytoplasma. Both the diseases are transmitted from coconut like many other diseases and pests of oil palm.

BSR disease of oil palm can cause potential yield and economic loss. During the initial period of planting of oil palm in India, BSR disease was not noticed much, however, the incidences are reported more and more during recent time mainly where under planting is done in the coconut gardens. Since the disease symptom appears at the later stage of infection, once it appears, it is difficult to recover and cure the palm. Accurate diagnostic technique, which is specific and readily adaptable to large scale testing for detecting *Ganoderma* in palms at an early stage of infection, would benefit decision-making for appropriate disease control.

With respect to diagnosis of the organism, some progress on development of precise technique for early detection of *Ganoderma* were reported through enzyme-linked immunosorbent assay using monoclonal antibody and PCR techniques elsewhere (Darmono, 2000; Utomono and Niepold, 2000). In India, ELISA with polyclonal antibodies is used so far as advanced technology. But ELISA with polyclonal as well as monoclonal antibodies is reported to be non- specific. PCR-based assay appears to be more specific. Keeping this in mind attempts has been made to standardize a protocol using *Ganoderma* specific primers to identify the fungi, which can finally be used as a regular detection tool. Primers were designed from the DNA sequences of *Ganoderma* sp., which codes for internal transcribed spacer (ITS) region of ribosomal RNA (Bridge *et al.*, 2000). For monitoring and diagnosis of the BSR disease in India the molecular diagnostic technique is being followed by NRCOP. Very small amount of tissue was used to detect these diseases by using the PCR technique. One pair of primers namely *Gan*1 (TTG ACT GGG TTG TAG CTG) and *Gan*2 (GCG TTA CAT CGC AAT ACA) was

used for detection of *Ganoderma*. A 167 bp band was observed in the PCR product in case of positive isolates. The result was confirmed using another pair of *Ganoderma* specific primers namely *Gan* ET and ITS producing an approximate 500bp band (Fig. 3) (Mandal *et al.*, 2006). The 500 bp fragment obtained using PCR amplification of *Gan*ET and *Gan*ITS were sequenced with an objective to identify the species. Mainly the sequence was matched with available database of NCBI site, and the most likely species in each case was found as *G. lucidum* or *G. applanatum* (Unpublished data). This confirms the spread of the disease is from coconut to oil palm. The main importance was that the oil palm plantations were treated with proper managament and control measures and hence the spread of the disease was controlled.

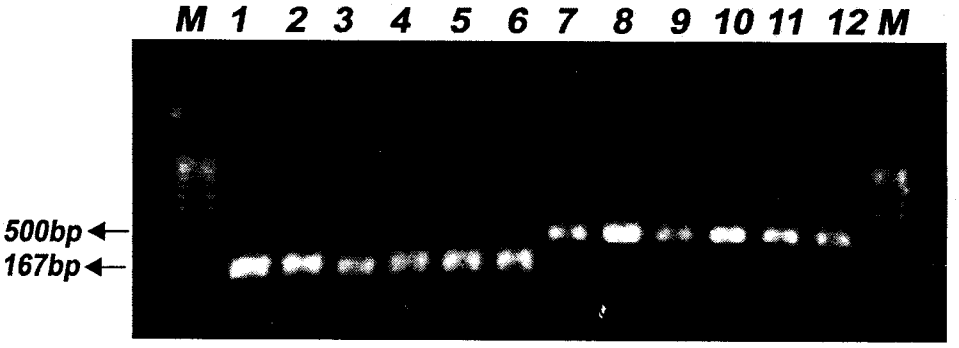



***Figure* 3: DNA from different *Ganoderma* isolates amplified using *Gan*1 & *Gan*2 & *and GanET & Gan ITS primers. Lane 1-6: DNA from GanO-01 to GanO-06 amplified using Gan*1 & *Gan*2 and Lane 7-12: same DNA amplified using *GanET & Gan ITS primers***

Spear rot disease (SRD) of oil palm is confined to Kerala state of India and caused by Phytoplasma. This lethal disease of oil palm, which causes complete arresting of the productivity, is of internal quarantine importance also. Root wilt disease of coconut and yellow leaf disease of arecanut have been proven as the source of inoculum for SRD through transmission studies (Kochu Babu, 1993). Diagnosis of the disease before symptom manifestation and molecular characterization would not only help in proper management of SRD of oil palm but also throw light on its relationship with root wilt disease of coconut and yellow leaf disease of arecanut.

With the advent of PCR based detection technology, the work has again been started recently to confirm the earlier studies and also to study the variation of the Phytoplasma of oil palm in different parts of Kerala state. Presently at National Research Centre of Oil Palm, different Phytoplasma specific primers are employed for diagnosis of the disease. Primers reported by Ahrens & Seemuller (1992) could amplify a fragment of

approximately 600bp specific to Phytoplasma. Work is in progress to develop a technique, which can be routinely used for the early detection of the disease.

**Bio-prospecting**

So far the discussion was on the betterment of the oil palm crop and its management. However, a new area of research is emerging, where the important products, metabolites, and mainly genes of oil palm can be utilized for producing high value products by other plant or organism. This aspect of bio-prospecting is going to gain more and more importance once the genome study of this crop, structural genomics and functional genomics of it is completed.

Already the reports of palm kernel oil, palm olein, crude palm oil and palm acid oil were used for the synthesis of poly (3-hydroxybutyrate-co-3-hydroxyhexanoate) [P(3HB-co-3HHx)] by a mutant strain of *Wautersia eutropha* (formerly *Ralstonia eutropha*) harboring the *Aeromonas caviae* polyhydroxyalkanoate (PHA) synthase gene. Palm kernel oil was an excellent carbon source for the production of cell biomass and P(3HB-co-3HHx) (Loo *et al*, 2005). Similarly it is demonstrated that inexpensive plant oils are excellent carbon sources for efficient production of PHA using Alcaligenes eutrophus strains (Fukui and Doi, 1998).

A Novel B-type gene from oil palm has been patented by Van der Linden *et al.*, (2006), which is claim to be the genetic sequences which could be used inter alia for the production of plants and, in particular, oil palm plants, which have modified phenotypes and/or which exhibits more highly desired characteristics such as, for example, male sterility or plants in which the sex ratio may be manipulated, and for the diagnosis and, preferably, elimination of the mantled phenotype. Similarly Abdullah and Kulaveerasingam (2002) patented molecular markers from oil palm peroxiredoxin gene, which can be used for embryogenic tissue to be detected *in vitro*. The early detection of embryogenic tissue enables non-embryogenic tissue to be discarded. This invention further contemplates a molecular marker comprising in one form a sequence of nucleotides encoding an antioxidant or in another form a sequence of amino acids defining a polypeptide having antioxidant activity. The antioxidant according to this aspect of the invention is particularly useful in tablet or cream form as an anti-ageing agent. The molecular markers of the present invention therefore also have uses in the inhibition or retardation of apoptotic processes. Such an effect has benefits in both plant and animal cells. The present invention further contemplates a promoter sequence preceding the molecular marker and its use in generating male sterile plants.

It has recently been shown that tocotrienols, which are produced mainly by oil palm, are the components of vitamin E responsible for growth inhibition in human breast cancer cells *in vitro* as well as *in vivo* through estrogen-independent mechanisms. Although tocotrienols act on cell proliferation in a dose-dependent manner and can induce programmed cell death, no specific gene regulation has yet been identified. However, it is suggested that tocotrienols are able to affect cell homeostasis, possibly independent of their antioxidant activity (Nesaretnam *et al.*, 2004).

It is understood that the lipase enzyme in oil palm is different from others and it is active at lower temperature. Oil palm fruits exposed to temperatures of 15°C and below showed a significant increase in free fatty acid (FFA) content in the mesocarp. This effect was most pronounced in fruits exposed to 5°C when FFA levels exceeding 70% of the total oil were observed. It is suggested that the activation of a lipase in the mesocarp can be achieved by low temperature stress. (Sambanthamurthi *et al.*, 1991). This unique enxyme can be produced by other organism for industrial use.

Similarly, Oil palm fruit yields the highest amount of carotenoids among all plant sources. Red palm oil extracted from the fleshy mesocarp of the oil palm (*Elaeis guineensis*) fruit, is the richest natural food source of β-carotene, having 400-700μg of β-carotene/g of oil compared to 2-50 μg/g in other yellow fruits and vegetables. Once the genes responsible for such high expression of β-carotene is elucidated and if the genes prove to be more efficient in producing carotenoids other than in oil palm system, they can be over expressed in other plants producing/not producing carotenoids or in microbial systems for production, extraction and commercialization, which may add to the current repertoire of carotenoids rich sources available for consumption.

In the conclusion we can emphasize the role of biotechnology for long term sustainable oil palm cultivation in India, where not only the improved variety or clones of oil palm would be generated with the capacity to produce higher bunch as well as oil yield, can also be successfully grown under resources limited conditions. Management of the crop, especially against biotic and abiotic stress, would be more efficient. Other than thealready established value added food and non food products from the oil as well as plantation and factory by-products and wastes, this plant can be the resource for several other product development in biological system, both in plant and in microbes. The process has already been on elsewhere, we also have initiated a humble beginning.

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