Oil Palm for Farmers' Prosperity and Edible Oil Security
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Oil Palm Biotechnology in India - Past, Present and Future

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ABSTRACT

Research on oil palm in India started during 1960 with the establishment of a research station at Thodupuzha by the Dept. of Agriculture, Kerala. Major achievement is the assemblage of more than hundred germplasm accessions, indigenous seed production and identification of superior hybrid combinations. Biotechnological intervention for oil palm improvement was attempted only during recent years. Oil palm being a cross pollinated crop with very long generation time, developing pure line is almost impossible. There is no common method of vegetative propagation applicable for this palm and hence, regeneration of an elite palm can be done only by tissue culture. A few protocols on *in vitro* regeneration of oil palm from seedling explants were reported but they are of not much commercial use since the starting materials were not from elite palms.

At NRCOP, research on the biotechnological aspects started very recently. A novel DNA extraction method was developed for oil palm, genetic diversity studies of the germplasm are in progress using both RAPD as well as SSR markers. Identification of shell thickness marker as a part of marker assisted selection is initiated. A small tissue culture facility is established to develop an indigenous protocol for *in vitro* regeneration of oil palm. It is realized that biotechnology can be applied as a main tool for oil palm improvement and the aspects which require major attention are as follows: Marker Assisted Selection (MAS) 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. Other than shell thickness, characters for MAS need to be prioritize and mapping population requires to be developed. Genetic diversity analysis using an authentic marker system needs to continue. Developing a tissue culture protocol for regeneration of mature palms is yet another area of importance, which can be used for mass multiplication of an elite *tenera*, raising separate stand of elite *dura* and *pisifera* palms and production of biclonal seeds, and also a base for transformation and development of transgenic oil palm. A few other aspects of biotechnology tools which can be adopted in future course are development of somaclonal variants for widening the variability, pollen culture for haploid development for pure line and *in vitro* conservation of oil palm.

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PAST

Oil palm, the highest yielding oil crop in the world has been introduced in India to meet the edible oil demand. Oil palm is cultivated in nine states of India of which Andhra Pradesh has the highest area under this crop. Though oil palm is grown as rainfed crop elsewhere, it has been introduced in India as an irrigated crop. Breeding programme started three decades ago with the *dura* parents of Malaysian origin available at Thodupuzha and pollen from NIFOR, Nigeria (Nampoothiri and Ravindran, 1996). Conventional breeding programme is continuing at this Centre. Major achievement are the assemblage of more than hundred germplasm accessions, indigenous seed production and identification of superior hybrid combinations. The success of oil palm cultivation under irrigated conditions eventually led to the research on several biotechnological aspects. Biotechnological research on this crop in our country has been started quite recently.

PRESENT

Molecular studies

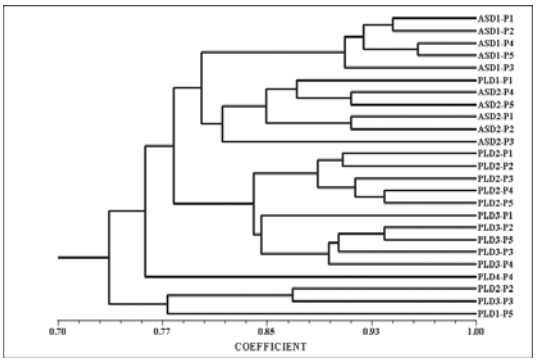
The first step in molecular biology of Oil Palm was the need for good quantity and quality of DNA. For the first time a novel method of DNA extraction has been developed (Jayanthi *et al. 2*004). It was found that DNA could be extracted from oil palm tender leaves without the use of a detergent. The same was tried with few commercial palms and other tree species. DNA extraction was possible without the use of any detergent in case of palms but not in case of other tree species. The genetic diversity of oil palm germplasm could not be assessed so far mainly because morphological data did not show significant differences among different accessions and it was realized that, molecular characterization of the germplasm was essential to know the extent of genetic diversity existing among the different oil palm germplasm in India.

Of the several markers reported to be used in oil palm (Ghesquiere, 1985; Jack and Mayes, 1993; Shah *et al*., 1994; Purba *et al*., 2000; Billotte *et al*., 2001) RAPD was most convenient, and the same marker was used for assessment of genetic diversity of different accessions.

Five of the *dura* germplasm accessions available in India, 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 (Mandal *et al*., 2004). The primers used were also evaluated for their usefulness to be employed for palm identification. 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. A total 167 bands from 33 primers, with an average of 5.06 bands per primer were observed. Total number of polymorphic bands were 111 accounting to 66.47% and the average number of polymorphic bands per primer was 3.36. Within the accessions, a maximum similarity of 0.958 was revealed between ASD1-P4 and ASD1-P5. Dendrogram derived from Cluster analysis (Fig.1) showed five distinct groups. All the palms from each accession formed separate group. Results from the present study revealed that no two palms were found completely similar in any of the five accessions mainly due to heterozygosity. Hence, it was felt that individual palms need to be characterized. Two utility parameters, Marker Index (MI) (Powell *et al.,* 1996) and Resolving power (R_p) were calculated for the primers.

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

Fig.1 : Dendogram generated by UPGMA analysis showing relationship among 25 palms from five different oil palm germplasm accessions using RAPD markers



Palode. This study by RAPD analysis using 24 random primers, which produced 141 reproducible bands, 95 of them were found polymorphic. Cluster analysis (by UPGMA method) showed six different groups, each consisting of palms from same accession, although no two palms from any accession were completely similar. 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).

Elaeis oleifera also known as American oil palm, has some desirable traits like better oil quality (in terms of unsaturation), disease resistance, slow growth rate etc. However, due to its low yield, this is used for inter-specific hybrid by crossing with common oil palm *(E. guineensis).* Twenty-three *oleifera* palms are, available at NRCOP, Palode, which have been collected from Malaysia and ASD Costa Rica. Characterization of these palms in terms of oil quality was felt very important. Simultaneously, the genetic diversity analysis was necessary before employing the palms for inter-specific hybridization as well as *oleifera* improvement programme. A high degree of genetic diversity was observed among the *E. oleifera* palms by RAPD analysis using eleven random 10-nucleotides primers. The maximum similarity was recorded between Eg-10 and Eo-11 (0.952) and minimum (similarity: 0.710) was between two pairs of palms (Eo-05 & Eo-08 and Eo-12 & Eo-21). The *oleifera* palms formed three major groups by UPGMA method cluster analysis. However, the two palms from ASD Costa Rican origin did not form any separate group though they were considerably similar (0.903).

Microsatellites or SSRs are stretches of randomly arranged short sequence motifs (ranging from two to six nucleotides), which are abundant and highly polymorphic in several eukaryotic genomes. DNA sequences flanking the microsatellite loci are known to be conserved, which can be used for designing suitable primers for amplification using PCR. This marker is more reproducible than that of RAPD, where as the ease of the technique was good as the latter one. Hence, the SSR marker was thought to be used for the genetic diversity study of oil palm.

Several oil palm genomic sequences, suspected to be microsatellite sequences are available in National Centre for Biotechnology Information site in the Internet hosted by National Institute of Health, United States of America. Two thousand and fifty such sequences were downloaded and primers were designed for 125 SSRs. Flanking sequences were used for designing primers and by using software 'OLIGOS' Version 6.2. Out of 80 pairs of primer sequences tested for their functionality, 74 pairs of primers were found functional. The primers would be used for genetic diversity study of the oil palm germplasm (Mandal *et al.*, 2006).

In the process of optimization of PCR reagent for SSR markers, it was found that a reduced concentration of Primers, Taq

Polymerase and dNTPs was enough for the amplification of oil palm microsatellites. The Primers concentration was reduced from 0.2µM to 50nM, Taq polymerase from 1U to 0.25U and dNTP (each) from 0.5µM to 0.25µM in a reaction volume of 25µL. Further the reaction volume was reduced from 25µL to 10µL since only a maximum of 10µl is loaded for gel electrophoresis. Cost economics was worked out taking into account the cost of the Taq polymerase, Primers and dNTP's. Using this optimized method the cost for 1000 reactions was Rs.920/- and it was around nine times lesser than the original cost (Jayanthi *et al.,* 2009).

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. Preliminary attempts have been taken to screen PCR primers and select the probable primers linked to the shell thickness. 'Bulk segregation' approach was adopted for the study and DNA isolation was carried out from 25 *dura* and 25 *pisifera* palms. All the palms were developed from same *tenera* x *tenera* crosses. DNA from all the 25 *dura*s and 25 *pisifera*s were pooled separately. 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. (Mandal and Pillai, 2005).

Tissue culture

Till recently there were no serious efforts to develop a tissue culture protocol for the multiplication of elite palms. A few attempts have been made, where in the regeneration protocol is reported from seedlings or embryos (Thomas and Rao, 1985; Anitha and Sajini, 1996; Rajesh *et al.,* 2003). Since there is no method of multiplication or propagation of an elite oil palm, because all the palms are heterozygous in nature and seed produced are segregating. It has a single growing apex and there are no methods of vegetative propagation of this plant like other horticultural crops. Natural clonal propagation is not possible since the axillary buds give rise to inflorescences either male or female. Hence *in vitro* regeneration of the mature elite palms or tissue culture is the only way of multiplication of a elite palm and hence is the most important aspect in oil palm improvement. Realizing the importance work on this aspect has been initiated at NRCOP.

FUTURE

Clonal Propagation: In India, after 25 years of research on oil palm breeding, we have selected a few elite palms which essentially need vegetative propagation. Hence, it is utmost important to develop an *in vitro* regeneration protocol of oil palm using mature palm as ortet so that clonal propagation of the selected elite palms will be possible. Bunch analysis and oil quality determination processes for the available germplasm are undergoing at National Research Centre for Oil Palm (NRCOP). Ortet would be selected on the basis of research results on these criteria. Agronomic traits like dwarfness, resistance to biotic and abiotic stress would also be incorporated in the programme after the germplasm screening and selecting elite palms for these required characters. Bi-clonal seed production is one of the objectives of clonal propagation parallel to the mass

multiplication of elite tenera palms. Identification of the abnormal clones at an early stage is very important as far as clonal propagation is concerned. (From in the) light of research works conducted all over the world in this aspect, DNA fingerprinting seems to be the most reliable techniques for testing clonal fidelity and hence these will be incorporated.

Marker Assisted Selection (MAS)

In the future thrust of marker based technology for oil palm improvement, completion of genetic diversity of all the palms is important and the work is in progress. Reliable markers are required for the study and the result would definitely help in breeding programme. Among the different traits to be considered for MAS, shell thickness for varietal identification is most important. So far no method is available, which can identify the three varieties (*dura, pisifera* and *tenera*) at an early stage and the undesirable palms are maintained due to unavailability of marker.

Similar to varietal identification, fruit type identification of *virescence (green* immature fruit) and *nigresence* (purple immature fruit) at early stage is very important to plant the right type of palm. *Virescence* character 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. Hence this trait is one of the important characters for marker-assisted breeding.

Oil quality is one of the major concerns in oil palm. Developing palms with better quality oil in terms of unsaturated fatty acids would help in more consumption of this oil since it would be healthier oil. *Oleifera* palms are already reported to be rich in unsaturated fatty acids (oleic acid) and selection of interspecific hybrids through MAS, would help breeder to develop palms with high yield and better oil. *Oleifera* also has the character of disease resistance, hence the disease resistance character of *E. oleifera* can be tracked by MAS and can be incorporated into the commercial species *E.guineensis* through resistance breeding programme.

Similarly the dwarfness of the palms is an important trait for increasing the economic lifespan of the crop. Height increment is the trait to be considered for MAS. Drought tolerance is one of the traits which is very important for MAS and developing a desirable variety.

***In vitro* conservation**

Most of the germplasm available in India are introduced from different countries. During last 25 years of oil palm research, we also have developed some materials indigenously. Conserving this entire germplasm either *ex vitro* or *in vitro* is utmost important. *Ex vitro* conservation in the field has its own limitation due to space containt and its exposure to natural vagaries of climate and other biotic factors. It is more important especially with a crop like oil palm and with the increased number of accessions. Hence, *in vitro* conservation is another priority area of oil palm biotechnology research to be taken up in future in the country.

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