Isolation of oil palm floral expressed sequence tags (ESTs) for the development of a floral cDNA chip

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Key words:EST, gene expression, floral development, sequence, cDNA

Introduction

Oil palm is an important crop in Malaysia and in 1997, palm oil contributed RM12.75 billion to the country's revenue. With the recent economic crisis, the foreign exchange earned by oil palm has become more important to the national economy. Oil palm crop production is related to weather conditions and poor crop is often related to drought conditions. During low rainfall periods male inflorescences are more predominant and there is a greater rate of inflorescence abortion. Inflorescences represent the first committed step in the formation of fruit and oil and therefore an understanding of the molecular biology of floral development is very important. In addition to this information about gene expression changes in response to environmental stimuli will provide ideas of interventional measures that can be taken to overcome this problem. Partial sequencing of randomly-selected cDNA clones to generate expressed sequence tag (ESTs) has become the method of choice for rapidly identifying new genes and characterizing transcript populations in tissue. When this project was implemented there were no genetic sequence resources for oil palm. Therefore, the generation of gene sequence resources for Oil Palm will contribute significantly to genetic improvement programmes. ESTs have also been used to construct RFLP lingkage maps and physical maps of the chromosomes. ESTs have also facilitated studies of gene expression and the fine structure of gene families. Fine mapping of oil palm genome is being carried out by PORIM and few other plantation companies in Malaysia. The data obtained from this EST project when coupled with the fine map will provide invaluable information for the improvement of Oil palm as a whole.

Materials and Methods

Construction of cDNA library

Total and poly(A)+ RNA were isolated from oil palm vegetative meristem, male and female flower. Three cDNA libraries were constructed according to the Uni-Zap cDNA Library kit (Stratagene) instruction.

Isolation of EST with Cold-Plaque screening procedure

The oil palm vegetative meristem, male and female cDNA libraries were hybridized with vegetative meristem, male and female flower cDNA probes respectively. The non-hybridising and weak signal clones were selected. *In vivo* excision of the clones was done and amplification of the inserts was carried out with T3/T7 primers using standard PCR conditions. In order to determine the spatial pattern of gene expression PCR products were run on 1.2% agarose gels, blotted onto nylon membranes and hybridized overnight at 68 C with cDNA probes. The membranes were then also hybridized to cDNA probes derived from oil palm young leaf tissue prior to being selected for sequencing.

Sequence analysis

Sequencing was performed with an automatic sequencer 377A with the dideoxy chain termination method (ABI PRISM 377 DNA sequencer, Applied Biosystems).

Results and Discussion

Three cDNA libraries of good quality were constructed from oil palm stage 3 female flowers, stage 7 female flowers and vegetative meristem. The average insert sizes of this cDNA library averaged over 1 kb in length with between 1-3% of non recombinant contaminating clones.

ii) EST generation

Over 5,000 clones were cored out from four cDNA libraries (oil palm male stage 3 flower library, stage 3 female flowers, stage 7 female flowers and vegetative meristem library). Only clones of above 500bp were subjected to sequencing to increase the probability of obtaining positive alignments with known sequences in the GenBank database. All of the clones picked for sequencing were identified from cold plaque screening and only cold or warm plaques were subjected to sequencing. This resulted in a reduction in the number of redundant sequences to between 5-

15% dependent on the library used. Reverse northern analysis was also preformed on most clones to confirm abundance as well as its tissue specific expression pattern. The majority of clones sequenced belonged to the low and medium abundance class of transcripts. The vast majority (>80%) of ESTs were expressed uniquely in the organ from which they were isolated with a minority expressed in other floral tissue as well. This indicates that most of the ESTs isolated appear to be unique to the floral organ and thus likely to be involved in unique features of these organs. When oil palm ESTs were compared with Arabidopsis and rice ESTs there was greater homology to Arabidopsis sequences. This was unexpected but could be a result of the poor annotation of the rice sequences as well as the lower numbers of rice EST sequences in the database compared to Arabidopsis. These EST clones fall into 12 categories based on their alignments to known genes that are as follows; hypothetical & unknown protein (30%), unknown protein (14%), cytoskeletal, structural & DNA repair (16%), energy & metabolism (7%), defense & stress (9%), photosynthesis (3%), signal transduction & communication (1%), protein kinase & protein phosphatase (4%), protease (1%), transporter (3%), transcription (10%), and translation (2%).

Conclusions

Three good quality cDNA libraries were successfully constructed from stage 3 oil palm female inflorescences, vegetative meristems and stage 7 female inflorescences. From these libraries a cold plaque technique was used to core over 5,000 plaques for analysis. Of these 4,000 were of sizes greater than 500 bp and were subjected to sequencing. Good quality sequences were obtained from 3,800 clones of which 3,200 represented non-redundant clones. Some of these clones were members of gene families and 2,800 unigenes were obtained from this project. Over 90% of these clones appeared to be unique to each organ (library) and are likely reflect the function/s of these tissues. These ESTs are a resource that has been used for the construction of an oil palm cDNA chip.

Benefits from the study

Three good quality cDNA libraries have been constructed from oil palm. 3,800 ESTs have been produced as a result of this study and have been used to support the production of oil palm cDNA chips. Three molecular biologists with MSc degrees and four biotechnologists with BSc degrees have been trained through this project. This project has validated the use of cold plaque screening as a means to identify quality non-redundant clones. Information developed in this project suggests that a large proportion of EST clones from floral and meristem tissue are specific to each organ/library and hence is likely to be involved in supporting the unique functions of these organs.

Patent(s), if applicable:

Nil

Stage of Commercialization, if applicable:

Nil

Project Publications in Refereed Journals:

Nil

Project Publications in Conference Proceedings

Choi MC, Ng, WH, Ho, CL., Sharifah, SA., Meilina Ong., K. Harikrishna, Tan SH. (2002). Characterisation and comparison of expressed sequence tags (ESTs) from cDNA clones of oil palm (*Elaeis guineensis*) flower and root by single-pass sequencing. In: Proceedings of the 12th Scientific Meeting of MSMBB. 22th - 24th May 2002, Saujana Hayatt, Kuala Lumpur.

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comparative genomics that are involved in floral development of oil palm and Arabidopsis. Plenary paper: The Plant Biology Winter Symposium, Pohang. Korea. 20-21 Jan 2003.

Graduate Research

Name of Graduate	Research Topic	Field of Expertise	Degree Awarded *(e.g. M.SC/Ph.D.)*	Graduation Year *(or expected)*
Choi Mei Chooi	The isolation and characterisation of EST clones from an oil palm floral cDNA library	Genomics	MSc	2003
Lee Yang Ping	EST analysis of an oil palm meristem cDNA library	Genomics	MSc	2003
Kwan Yen Yen	The isolation and analysis of oil palm MADS box genes and ESTs from ovules.	Genomics	MSc	Expected end 2003

IRPA Project number: 01-02-04-0453
UPM Research Cluster:BAB
Project Leader Harikrishna Kulaveerasingam