

Exploitation of Gene Rich Sequences for Improved Oil Palm Productivity

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ABSTRACT

The Malaysian Palm Oil Board (MPOB) in collaboration with Orion Genomics (USA) initiated a modest effort to selectively sequence certain regions of the oil palm genome in 2004. The sequences generated touched approximately 95% of functional genes of all four oil palm varieties (dura, tenera, pisifera and oleifera). The sequences have been extensively exploited to develop simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) molecular markers. The markers in turn have been used to generate genetic maps. Genomic loci linked to important agriculture traits such as shell gene, fruit color, fatty acid composition and yield components have been located on the genetic maps. The sequences have also been used to generate oil palm DNA Chips, which have proven useful in identifying gene signatures associated with tissue culture amenity. The existing sequence data have also been used to develop experimental transgenic palms through genetic engineering. These experimental palms have traits such as good oil quality (unsaturated oil) and also the ability to produce bioplastics. The transgenic palms are still in the experimental stage in the biosafety nursery.

BACKGROUND

The Malaysian Palm Oil Board (MPOB) in collaboration with Orion Genomics (USA) initiated a modest effort to selectively sequence certain regions of the oil palm genome as far back as 2004. The strategy chosen was to apply a sequencing technology, named as *GeneThresher*TM, that allows preferential selection of those regions of the genome that encode genes and their regulatory regions (Rabinowicz *et al.*, 1999). The method makes use of the fact that plant genes are usually hypomethylated while the repetitive regions are methylated (Budiman *et al.*, 2005). Using certain bacterial strains, the methylated repetitive sequences are “threshed” or removed, and are not sequenced, while a much smaller hypomethylated genespace is sequenced leading to comprehensive gene discovery at a greatly reduced cost. This allows comprehensive gene coverage to be achieved quickly and rapidly. Through this effort MPOB successfully generated over 400,000 genomic sequences from four oil palm varieties (*dura*, *tenera*, *pisifera* and *oleifera*). The sequences represent approximately 95% of the functional genes in oil palm.

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EXPLOITING THE SEQUENCES

MPOB's focus since 2005 has been to exploit the existing sequence data. As a result of exploiting the existing data, MPOB has managed to generate 10 patents related to important agronomic traits. Among the patents are markers for fruit color, absence/presence of shell and traits to improve yield. Although MPOB's research has given rise to several products, the actual commercialization will take a few years to be realized due to the long breeding cycle of oil palm (8-10 years). It is for this reason that MPOB is very cautious about making announcements and claims in this area.

The progress achieved by MPOB in exploiting the existing sequence data can be described as follows:

i. Development of bioinformatics structure

The PalmDNABase, a management and analysis system for DNA data generated through various approaches and techniques, was developed to facilitate the automation of storage and handling of sequence data and information. The database has the ability to analyze and store information and analysis data systematically. To date, a total of 430,000 sequences have been stored.

ii. Development of oil palm genetic maps and identification of markers for agronomic traits

MPOB has developed comprehensive genetic maps for the oil palm. These genetic maps help facilitate the positioning of the genes controlling the important agronomic traits. Based on these maps, genetic markers that have the potential to predict for the following traits have been identified:

- Oil palm fruit colour
- Presence/absence of the shell
- Oil quality

These molecular markers are currently being used in oil palm breeding to produce new oil palm varieties with higher oil yield. However, it is important to emphasize here that a long period is required to produce these new and improved varieties. The oil palm is a perennial crop, where one breeding cycle takes about 10 years. At a minimum, one breeding cycle is required to produce a new variety.

iii. Development of oil palm DNA Chips

Apart from selectively sequencing certain regions of the genome, there is also a research project aimed at generation of expressed sequence tags (ESTs). ESTs generated from present research programmes provide the framework for large-scale functional analysis of thousands of genes via the use of DNA Microarray technology or DNA Chip. The oil palm DNA chip can then be used to bridge the gap between sequence information and functional genomics and thus provide information on genome-scale sampling of gene

expression patterns. To date, about 8,000 oil palm genes have been printed and being actively used to understand gene expression during oil palm tissue culture.

iv. Increasing the efficiency of the tissue culture process

MPOB has already identified oil palm clones that have the potential to produce 10 tonnes oil per hectare per year. In an effort to increase the efficiency of the tissue culture process, MPOB is exploiting the existing sequence data. Molecular markers that can help predict the ability of a mother palm to be cultured have been identified. These molecular markers are currently being tested in collaboration with members of the industry such as AAR, United Plantations and FELDA.

MPOB is also using the existing sequence information to understand the abnormality problem observed in some clones generated by tissue culture. In collaboration with several renowned institutes from overseas, MPOB has successfully provided scientific explanations for the abnormality seen in the tissue culture clones. The information will also be used to develop molecular markers to predict whether a clone will produce a normal palm or otherwise.

v. Development of transgenic oil palm

a) Production of Transgenic Oil Palm

The existing sequence data have also been used to develop experimental transgenic palms through genetic engineering. These experimental palms have the following traits:

- good oil quality (unsaturated oil)
- ability to produce bioplastics

The transgenic palms are still in the experimental stage in the biosafety nursery and have been shown to produce the expected products. Molecular analysis as well as analysis of the traits (fatty acid and bioplastics) in oil palm and model crops has demonstrated the integration, expression and production of the products. The field trials will only proceed after the Ministry of Natural Resources and Environment has given its approval following the implementation of the Biosafety Act.



Figure 1: Transgenic oil palm biosafety nursery

b) Isolation of Constitutive Promoters

Isolation of promoters is a very important aspect of genetic engineering research for oil palm. Promoters are required to control the expression of genes introduced into oil palm. Information obtained from the DNA Chip program led to the identification of 50 clones showing constitutive expression. Further validation via reverse northern and northern analyses, conclusively identified a few gene clones showing constitutive expression. Full-length gene and promoters for two of these clones were successfully isolated from the *GeneThresher* data. Transient expression using these promoters showed *gusA* gene expression in all the tissues tested demonstrating the constitutive nature of the promoter.

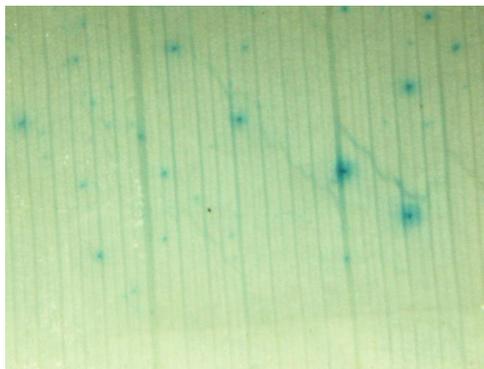


Figure 2: *gusA* gene expression on oil palm leaf using constitutive promoter

The available sequences are still insufficient to enable the achievements of all future targets. It is for this reason that MPOB initiated the oil palm whole genome sequencing project in January 2009.

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