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EXPLOITING SYNTENY BETWEEN OIL PALM AND RICE TO FIND MARKERS MORE CLOSELY LINKED TO SELECTED TRAIT

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ABSTRACT

*The African oil palm (*Elaeis guineensis*) and rice (*Oryza sativa*) are important economic crops. The rice genome has been more extensively characterised and studied compared to oil palm. GBrowse, a bioinformatics tool for visualising genomic and other sequences along a reference genome was applied in this study. Herein, we report the use of model organism* Oryza sativa *as a reference genome to map 75 463 genomic* E. guineensis *sequences. A subset of 328 oil palm sequences that aligned well to the 12 rice chromosomes was shortlisted for further analysis. Of these 328 genomic sequences, 261 contained microsatellite motifs and were apt for primer design. A total of 208 Simple Sequence Repeats (SSR) were selected to screen for polymorphism in a subset of palms from a selfed Nigerian* tenera *mapping population (T128), whereby, 67 SSR were found to be polymorphic. Thirty-eight of the polymorphic markers were mapped onto the genetic map. In comparison to the 12 rice chromosomes, six SSR from the oil palm linkage group 1 (LG1), containing the shell gene (SH) locus were identified on chromosomes 2 and 5 of rice. The order of these six SSR on linkage group 1 was mostly similar to that in chromosomes 2 and 5 of rice. The study provided evidence on synteny between oil palm and rice and facilitated the mapping of a marker closer to SH locus.*

Keywords: GBrowse, oil palm, rice, SSR, mapping.

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INTRODUCTION

Sequence information of organisms is rampant in public databases and escalating yearly. The genome sequences of nearly two dozen plant species are publicly available to date (Buell and Last, 2010). Thale cress or notably known as *Arabidopsis thaliana* and rice are the two gold standard plant reference genomes that have been extensively studied. These two plant models are routinely used as the forefront models for dicot and monocot species. The availability of complete sequences of these two plant models has helped revolutionise the understanding of other crop plants as well (Xu *et al*., 2005), including oil palm (*Elaeis guineensis*), where the genome is only now being extensively studied.

The Generic Genome Browser (Stein *et al*., 2002) being the proverbial bioinformatics tool is useful in visualising genomic features along reference sequences such as that of *A. thaliana* (Arabidopsis Genome Initiative, 2000) and *Oryza sativa* (The International Rice Genome Sequencing Project, 2005). Commonly known as GBrowse, this tool is part of the Generic Model Organism Database Toolkit, which is designed to manage and explore genome-scale biological databases. This tool is implemented via a web-based application that

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displays genomic annotation and various functional features. With the advent of GBrowse, comparative genomics, comparative mapping or chromosome mapping is feasible (Shimamoto and Kyozuka, 2002; Yu *et al*., 2004; Zhuang *et al*., 2008).

Comparing information between plant genomes particularly for species belonging to the same family such as the Brassicaceae, Poaceae, Fabaceae and Solanaceae (Paterson *et al*., 2000) is gaining importance. Moreover, comparative approaches of dicots (thale cress, soyabean, potato) and monocots (rice, maize, barley, wheat) have provided the impetus to exploit well characterised sequences to understand gene structure and organisation in non-model crops, such as oil palm. Comparative mapping uses DNA markers to establish the syntenic relationships between genomes of different species, families and genera (Fulton *et al*., 2002; Krutovsky *et al*., 2004; Varshney *et al*., 2005; Lohithaswa *et al*., 2007).

The available oil palm sequences have yielded significant similarities to the sequences of rice (Jouannic *et al*., 2005; Elyana *et al*., 2008). By using cluster of orthologues groups (COG), a bioinformatics tool that is able to distinctively characterise gene sets, particularly of closely related genes found in different organisms, Willies *et al*. (2008) found that the recognisable functions of the oil palm Expressed Sequence Tags (EST) were consistent with those from the rice genome. Ravigadevi *et al*. (2009) also reported that EST sequences had significant similarity to monocot plants such as rice, maize and wheat. Comparative mapping with rice is expected to provide new insights, knowledge and opportunities in understanding the genome of oil palm.

The first objective of this study was to exploit GBrowse for comparative mapping between genomes of oil palm and rice. The idea is to find rice sequences that align well with oil palm sequences, including those sequences that were mapped on an existing oil palm genetic map. Individual oil palm linkage groups could be aligned to rice chromosomes. This information can be used to further saturate regions of interest on the oil palm genetic map.

Using GBrowse, the oil palm EST (Low *et al*., 2008) and genomic sequences (Budiman *et al*., 2005) were aligned and mapped to their syntenic equivalents in the rice genome assembly. The EST that mapped to the rice genome had been previously converted to restriction fragment length polymorphism (RFLP) markers to generate a genetic map for a selfed Nigerian *tenera* palm T128 (Rajinder, 2005). This population segregates for the shell gene (SH), whose location was also determined on the genetic map. The shell trait is important as the oil palm has three fruit forms, namely *dura, tenera* and *pisifera*. These three fruit forms vary in their shell thickness, with *dura* having a thick shell of 2-8 mm, *tenera* which is a hybrid of *dura* and *pisifera* produces fruits with an intermediate thickness of 0.5-3 mm and *pisifera* has no shell (Hartley, 1988; Rajinder and Cheah, 2005). The higher mesocarp content in *tenera*s (hybrids) makes them the material of choice for commercial planting. Although the *pisifera* can have mesocarp content of 95%, they are usually female sterile and bunches can abort during development. Therefore, *pisifera*s are not grown as commercial crop. In 2000, Moretzsohn *et al*., worked on a single controlled cross (*tenera* x *pisifera*) and identified two RAPD markers which mapped on both sides of the SH locus on linkage group 4 with a distance of 17.5 cM and 23.9 cM respectively. Billotte *et al*. (2005) and Rajinder (2005) reported AFLP markers that were at a distance of 4.7 cM and 8.0 cM away from the SH locus in their respective oil palm mapping populations. The ideal marker for use in breeding should be much closer (Madan *et al*., 1997) to the SH locus.

The oil palm EST sequences have already been exploited for use as RFLP markers and mapped on the T128 linkage map. As such, the second objective was to identify Simple Sequence Repeats (SSR) within sequences with synteny to rice and map these SSR markers onto the existing oil palm genetic map. Among the numerous types of markers available, SSR are preferred. SSR markers are easy to use, reproducible, show co-dominant inheritance, multi-allelism and are PCR (polymerase chain reaction) based. SSR have been extensively used as genetic markers (Ghislain *et al*., 2004; Zhang *et al*., 2008; Zhao *et al*., 2008) and have also shown to be linked with genes involved in development, regulation and genome evolution (Lawson and Zhang, 2006; Tranbarger *et al*., 2012). SSR has been employed successfully to understand phylogeny of palm taxa (Billote *et al*., 2001; Rajinder *et al*., 2008), develop oil palm genetic maps (Billotte *et al*., 2005; Rajinder *et al*., 2009) and for DNA fingerprinting (Rajinder *et al*., 2007). Furthermore, by mapping SSR from sequences that have synteny in the rice genome, comparative mapping with the well characterised and studied monocot is possible.

MATERIALS AND METHODS

Plant Materials

Controlled self-pollination of the Nigerian palm T128 was used to generate the mapping population consisting of 104 palms.

DNA Extraction

DNA extraction of leaf samples was carried out essentially as described by Rajinder (2005).

Mapping of Oil Palm Sequences

A total of 425 240 oil palm sequences with an average length of 606 bp from both the EST (25 118 sequences) and genomic (400 122 sequences) libraries were used in this preliminary study. Firstly, the two complete genomes, namely *A. thaliana* (TAIR 6.0) and *O. sativa* (IRGSP Build 4) were downloaded from their respective sites. Subsequently, oil palm sequences that mapped to *A. thaliana* (5 chromosomes) and *O. sativa* (12 chromosomes) were loaded onto a GBrowse server. The oil palm sequences that aligned to the selected reference sequences were mined for SSR.

Primer Design

MISA (MicroSAtellite identification tool) as described by Thiel *et al*. (2003) was used to identify and localise microsatellite markers in the oil palm sequences. Next, primer pairs were designed using the Primer3 program (Rozan and Skaletsky, 2000).

SSR Analysis

The SSR markers were first used to screen a subset of the mapping population (13 palms including parental palm, T128). The polymorphic markers were then used to genotype the mapping population, comprising of 104 palms as described by Rajinder (2005). The genotyped SSR data were scored accordingly to the segregation ratio expected in the F_2 progeny. The two distinct segregating ratios observed were 1:2:1 and 3:1 for the co-dominant and dominant profiles exhibited by the respective SSR.

Linkage Analysis

Linkage analysis was performed using JoinMap version 4.0 (Ooijen, 2006). The polymorphic marker set was combined with a previous data set consisting of RFLP and AFLP markers that mapped on the linkage group (Rajinder, 2005). The map distances (cM) were attained using the Kosambi mapping function. A logarithm of odds (LOD) threshold of 6.5 was used to assign the markers into linkage groups. The information from the new T128 linkage map, particularly linkage group 1 (LG1), containing the SH locus was compared to the rice chromosomes. This was viewed graphically using the MapChart software (Voorrips, 2002).

RESULTS AND DISCUSSIONS

Mapping Oil Palm Sequences to the Rice Genome via GBrowse

Information from model species will facilitate the understanding and interpretation of gene function and genetic map development in other crops. The complete sequencing of the rice genome has given an opportunity to exploit the genome information of this monocot to help understand the genome organisation in oil palm.

In this preliminary study, genome mapping of 425 240 oil palm sequences was carried out on the two complete plant genomes, *A. thaliana* and *O. sativa*. The oil palm sequences were derived from 25 118 EST and 400 122 genomic sequences, respectively. The mapping of these sequences revealed that the oil palm sequences aligned better to monocots compared to dicots (*Table 1*). As such, priority was to focus on comparative mapping with rice in this study.

Since the EST sequences have been utilised as RFLP markers on the T128 genetic map (Rajinder, 2005), hence, the genomic sequences that mapped to the rice genome was prioritised. Of the 400 122 genomic sequences, about 18.86% aligned well to the rice genome (*Table 1*).

GBrowse was designed to visualise oil palm sequences mapped to the model organisms and display a set of protein or nucleotide features. The GBrowse search functionality is very flexible and easy to use. It is able to search for an oil palm sequence by its clone accession number, contig name, genetic marker name or any names that was configured into the GBrowse database, fetch the region of the genome (*O. sativa*) that spans the oil palm sequences (*E. guineensis*) and displays the aligned sequences as reflected in *Figure 1*. The detailed view of multiple chromosome tracks of the alignments with similarity values between *O. sativa* and *E. guineensis* were displayed. The positions of the oil palm sequences aligned on the rice chromosomes were also featured.

By means of utilising GBrowse, a subset of 328 sequences from the 75 463 genomic sequences were selected from regions of interest on the genetic map (to increase the oil palm map density) that aligned with high confidence to the 12 rice chromosomes. Of these 328 sequences, a total of 261 genomic sequences contained microsatellite motifs and were suitable for primer design. Only 67 of the genomic

TABLE 1. PERCENTAGE OF OIL PALM SEQUENCES ALIGNED TO THE REFERENCE GENOMES

Oil palm sequences	*A. thaliana*	*O. sativa*
	(%)	
All sequences	14.10	20.27
EST sequences	27.02	42.80
Genomic sequences	13.29	18.86

Note: EST - Expressed Sequence Tags.

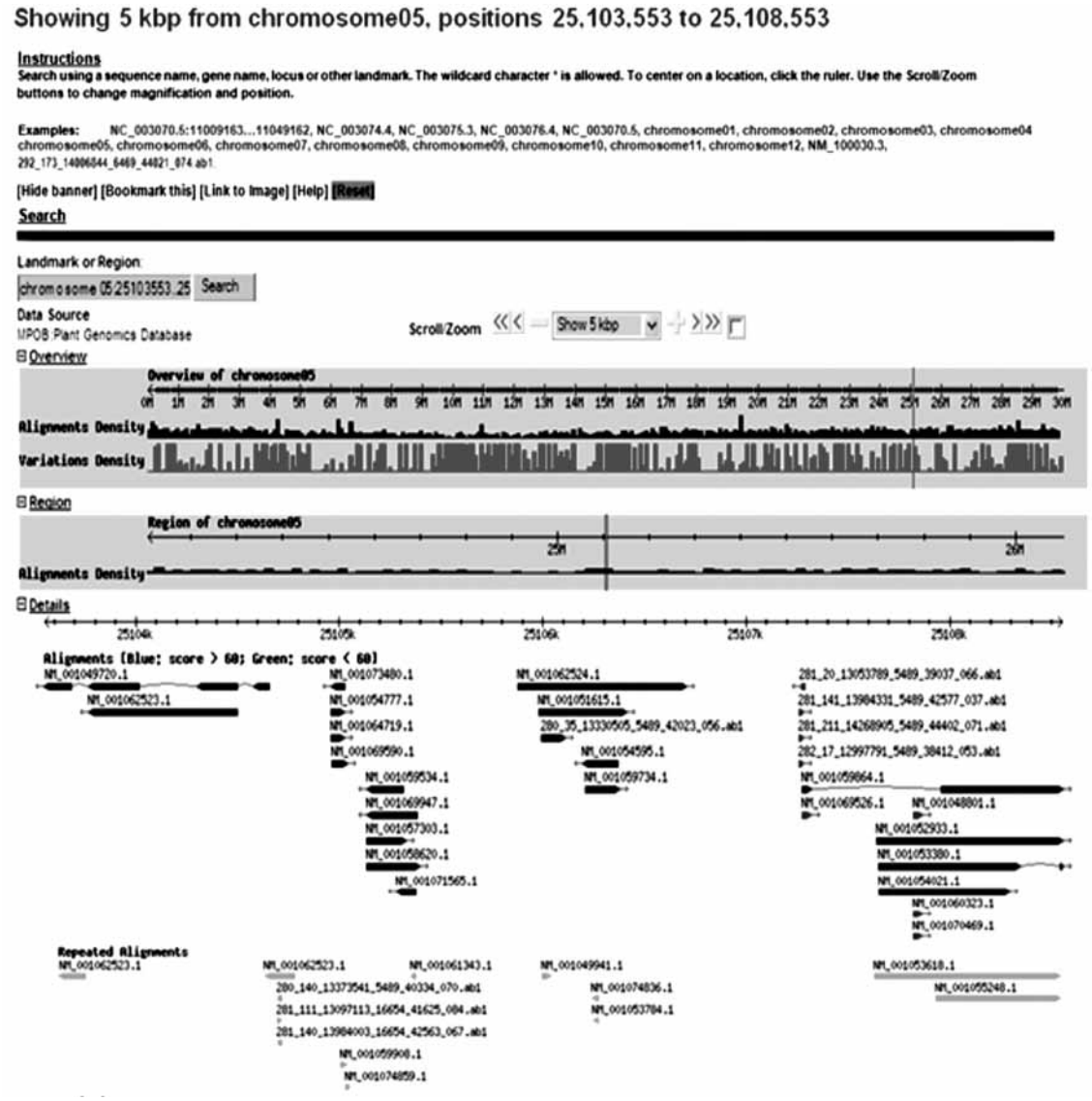

Figure 1. An overview of the aligned oil palm sequences with an O. sativa *chromosome.*

oil palm sequences did not have SSR. In this preliminary study, 208 SSR primer pairs (an SSR for each of the 208 genomic sequences) were randomly selected to screen for polymorphism in a subset of the mapping population (13 palms including parental palm, T128).

SSR Markers on the T128 Linkage Map

The screening of the selected 208 genomic SSR primers revealed 67 polymorphic amplicons. About 32.2% of SSR markers used were informative. The 67 informative SSR loci were genotyped on the T128 mapping population consisting of 104 palms. Subsequently, using the JoinMap (version 4.0) software, 38 SSR markers were successfully mapped on the T128 genetic map (*Table 2*). The SSR markers were mapped on 14 of the 16 linkage groups. Only linkage groups 9 (LG9) and 11 (LG11) did not contain the SSR markers. LG1 has the most number of markers mapped and this was very useful in the present study.

TABLE 2. 38 SIMPLE SEQUENCE REPEATS (SSR) MAPPED ON THE LINKAGE MAP OF T128

Linkage group	No. of SSR	Linkage group	No. of SSR
1	6	9	-
2	4	10	4
3	1	11	-
4	5	12	3
5	3	13	2
6	1	14	2
7	3	15	2
8	1	16	1

Selection of Genomic SSR

Previously, Rajinder (2005) had reported mapping of the SH locus on LG1 using the same mapping population. Of the 38 SSR markers successfully mapped, six were located on LG1 containing the SH locus. It was observed that these six SSR were aligned to two different chromosomes of rice.

Rice chromosomes 2 and 5 were correlated with the six SSR markers on LG1. It was observed that four of these SSR aligned to chromosome 2 and two SSR to chromosome 5 of rice. It would appear that chromosomes 2 and 5 of rice were associated with LG1 containing the SH locus. The markers order on the rice chromosomes 2 and 5 were mostly consistent with the order of the markers on LG1, except for marker sMg00203. The marker sMg00203 mapped on chromosome 2 of rice was located at a distance of 10.9 cM from the SH locus, in LG1 (*Figure 2*), which is closer to marker sMg00155 in oil palm. In rice, sMg00203 was placed closer to marker sMo00028.

The six SSR markers on LG1 were distributed from 38.0 cM to 88.4 cM, whereby, the SH locus was located at 45.5 cM. The closest SSR marker, sMg00230, to the SH locus was at a distance of 2.6 cM. The distance was reduced compared to the AFLP marker (4.7 cM) reported by Billotte *et al*. (2005) in their mapping population. More importantly, the preliminary study helped to identify and exploit synteny between oil palm and rice. This information was used to find markers more tightly linked to the SH locus. Efforts are on-going to identify the shell gene itself. This would be an important accomplishment considering the importance of a marker tightly linked to the SH locus in oil palm breeding and commercial seed production. The similarity of some of the mapped SSR containing sequences to *O. sativa* is shown in *Table 3*.

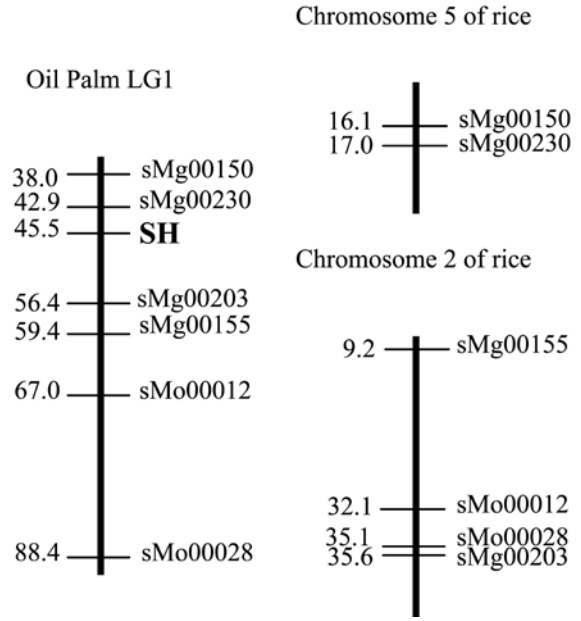

Figure 2. Six simple sequence repeats (SSR) markers aligned to chromosomes 2 and 5 of rice and mapped on the oil palm LG1.

TABLE 3. SIMILARITY OF SOME MAPPED SIMPLE SEQUENCE REPEATS (SSR) CONTAINING SEQUENCES TO RICE CHROMOSOME 5

Rice chromosome	SSR markers	SSR type	Description of clones (Blastx)	E-value
5	sMg00150	$(TA)_{14}$	Os04g0501800 [*Oryza sativa* Japonica Group] - gi | 115459264 | ref | NP_001053232.1	2e-18
	sMg00230	$(CT)_{17}$	Hypothetical protein OsI_07650 [*Oryza sativa* Indica Group] gi | 125539885 | gb | EAY86280.1	2e-08

CONCLUSION

Comparative analysis of *E. guineensis* and *O. sativa* genomes has yielded some insights on the synteny between the two crops. Using the rice genome as a plant model, we were able to obtain SSR in the same linkage group as the SH locus, LG1. This shows that the two crops to some extent, share synteny which can be exploited in oil palm research. As demonstrated here, if a trait is located on the genetic map, the complete reference genome of rice could prove useful in locating markers closer to the trait concerned. The significant result in this study was that SSR markers mapping closer to the SH locus were identified within a short period of time. This also provides an opportunity to further leverage on the rice genome to find markers more tightly linked to the SH locus. This has also inspired us to use the same approach to search for markers linked to other traits of interest, by using rice as the reference genome.

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