International Journal of Oil Palm Vol. 9 (1&2), 2017

REVIEW ARTICLE

Oil Palm breeding strategies through molecular and genomics technologies: status and way forward

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Received: July, 2017   Accepted: June, 2018

ABSTRACT

The collection and exploitation of germplasm material is considered a major factor in contributing towards improvement of oil palm industry. Conventional breeding can take several years, which greatly hampers rapid and efficient progress in the selection of individuals. Various molecular biology techniques are available today for detection of genetic variability and for establishing genetic similarity relationships among individuals and can be used in various applications in breeding programs. These modern genomics tools allow substantial knowledge to be attained on the biological traits of a species which can be exploited in improving productivity and obtain better product quality. The recent advances in genome sequencing through next generation sequencing (NGS) technologies provide opportunities to develop millions of novel markers, as well as the identification of agronomically important genes. The present review focused on molecular approaches for improving breeding capabilities in oil palm breeding programmes.

Key words: Oil palm, tenera, genomic selection, molecular breeding

INTRODUCTION

Improved oil palm varieties with higher yields, good oil quality, and compact in architecture, better adaptation to climate change and higher tolerance to diseases have been prioritized to meet future demands of the oil palm industry. The collection and exploitation of germplasm material is considered a major factor in contributing towards improvement of oil palm industry. Oil palm breeding programs are characterized by using the reciprocal recurrent selection scheme which uses two dura and pisifera-type starting populations to make the crossings and progeny testing of which the best parents are chosen and tenera-type seeds are produced. Subsequently, new populations are generated and the cycle is repeated (Corley and Tinker, 2003). Same female parental stock, Deli *dura*, with some introgressions and are combined with three male parental stocks (pisiferas), the Avros, La Me and Yagambi (Bakoume 2007 and Cochard, 2009). This narrow genetic base has driven oil palm breeders to play greater importance on genetic resources of the species to increase the genetic variability in breeding programs.

The breeding scheme primarily involves a reciprocal recurrent selection scheme (RRS) which has been adapted by a number of oil palm breeders. In this scheme, the *dura* and *pisifera* genotypes are kept as distinctly separate base populations. In the form of hybrids (*tenera*), the heterosis effect is obtained by crossing origins with complementary characteristics. The performance of inter-origin crosses is attributed to the additive effect of favorable genes combination from the parents. It was reported that the RRS scheme has increased oil yield by almost 18% per cycle compared to the base population (Rajanaidu et al. 2000). Introgression of current materials with selected materials could be handful way to broaden their genetic diversity. Combining ability is essential to identify superior parents for hybrid seeds production. There are two types of combining abilities, general combining ability (GCA) and specific combining ability (SCA). GCA plays important role in the identification of parents for the development of superior genotypes while SCA provides information about the performance of hybrids (Cruz and Regazzi 1994). The AVROS *pisiferas* were known to have high general combining ability. In oil palm, efforts have been made to exploit the GCA and SCA among parents to increase fresh fruit bunch (FFB) yield, oil-to-bunch ratio (O/B) and kernel-to-bunch ratio (K/B) by 42%, 18% and 29% (Breure and Konimor 1992; Dumortier and Konimor 1999 and Rafi et al. 2001). Okwuagwu et al. (2008) reported that cross of Deli *dura* x *tenera* breeding in Nigeria indicated that high estimates of genotypic coefficient of variation, heritability and genetic advance recorded for the bunch yield traits. Noh et al. (2012) evaluated the performance of 11 oil palm AVROS pisiferas and observed low genetic variability among pisifera parents for most of the characters indicating uniformity of the pisifera population and suggested that the low variability is due to the small population size of AVROS pisifera from which they have been derived.

Conventional breeding can take several years, which greatly hampers rapid and efficient progress in the selection of individuals. Various molecular biology techniques are available today for detection of genetic variability and for establishing genetic similarity relationships among individuals and can be used in various applications in breeding programs. These modern genomics tools allow substantial knowledge to be attained on the biological traits of a species which can be exploited in improving productivity and obtain better product quality. Complementation of conventional breeding technique with novel approaches from biotechnology will accelerate the progress in oil palm improvement (Ramli et al. 2016 and Murphy 2014). Though there are some reviews available (Babu and Mathur 2016; Kumar et al. 2018), the present review focused on recent trends in molecular breeding and next generation technologies in oil palm.

Marker-Assisted Selection and QTL mapping

Marker assisted selection has been carried out in the progeny, which allows the early selection of desired progeny. DNA markers such as restriction fragment length polymorphism (RFLP), random amplification of polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), single sequence repeat (SSR), and single nucleotide polymorphisms (SNPs) have been identified and applied to improve breeding of oil palm. Simple Genetic linkage information is potentially a very powerful tool for accelerating oil palm breeding through marker-assisted selection. Ritter et al. (2004) used three primers for differentiation of dura, pisifera and tenera forms. Moretzsohn et al. (2000) constructed a linkage map with RAPD markers and identified two RAPD markers to be linked on both sides of the sh+ locus on linkage group 4. Sequence Repeats (SSR) have been efficiently used to study the genetic structure of oil palm (Billotte et al. 2007 and Singh et al. 2008), for varietal identification (Norziha et al. 2008), genome mapping and QTL detection for molecular marker assisted selection (Billotte et al. 2010). Fatty acid composition is an important agronomic trait which is associated with oil quality and QTL mapping for this trait using molecular markers will speed up the production of new and improved oil palm planting materials. Singh et al. (2009) described the first successful detection of QTLs for fatty acid composition in oil palm. The genetic linkage maps enriched with simple sequence repeat (SSR) markers were developed for dura (ENL48) and pisifera (ML161), the two fruit forms of oil palm to study the tissue culturability and identified two quantitative trait loci (QTLs) associated with callusing rate and embryogenesis rate (Ting et al. 2013). Lee et al. (2014) constructed a linkage map of oil palm using 2795 co-dominant DNA markers and mapped a major QTL for stem height on the linkage group 5 and stated that the markers flanking the QTL could be used in the selection of dwarf trees at the seedling stage, thus accelerating the breeding for shorter trees. Babu et al. (2017) developed *Sh* gene specific primer pairs using 300 genomic, 8 genic SSR markers and identified one cleaved amplified polymorphic site (CAPS) marker for differentiation of oil palm fruit type and suggested that selection and distribution of desirable high yielding tenera sprouts to the farmers could be possible at seedling stage instead of waiting for 4–5 years and saves a lot of land, time and money which will be a major breakthrough to the oil palm community. Bai et al. (2017) constructed a high-density linkage map with 1357 SNPs and 123 microsatellite markers to provide a basis for fine mapping of QTL and improve the assembly of the genome of oil palm and found four QTLs for oil to bunch (O/B) and oil to dry mesocarp (O/DM) on LG1, LG8, and LG10 in a F1 breeding population and also identified DNA markers associated with these traits. They have suggested to pyramiding the identified QTL with beneficial genotypes associated with oil content traits using DNA markers has the potential to accelerate genetic improvement for oil yield in the breeding population of oil palm.

Genomic selection

Genomic selection (GS) uses genome-wide markers as an attempt to accelerate genetic gain in breeding programs for perennial crops such as oil palm, which have long breeding cycles; Genomic selection (GS) is an efficient method of marker-assisted selection to improve quantitative traits using markers distributed across the entire genome (Meuwissen et al. 2001).
In traditional breeding schemes, the progeny tests enable highly accurate selection, but the low rate of genetic gain is the main drawback. The difficulty and costs associated with long term evaluations of progenies limit the number of individuals evaluated, resulting in low selection intensity. The ultimate goal of GS is to expedite the breeding progress by maximizing the genetic gains per generation. In this context, the potential of GS for palm oil yield is high, and several previous researchers also report the potential application of GS in oil palm (Wong et al., 2008 and kwong et al. 2017). Pootakham et al. (2015) reported the efficiency of this approach for quantitative trait loci (QTL) detection in oil palm. Cros et al. (2017) reported the possible application of GS in oil palm by conducting genomic pre selection in the parental populations prior to progeny tests, which increased selection intensity for yield components thus improving the performance of commercial hybrids using GBS and suggested further research to increase the benefits from GS, which should revolutionize oil palm breeding.

Genome-wide association studies (GWAS)

The CK et al. (2016) performed GWAS for oil-to-dry-mesocarp content on 2045 genotyped tenera palms using 200K SNPs and found that 80 loci were significantly associated with oil-to-dry mesocarp yield (P d" 10-4), and three key signals were found. Ithnin et al. (2017) conducted the multi-locus Genome-Wide Association Studies (GWAS) and identified 19 quantitative trait loci (QTLs) for 8 traits and further reported the potential application of GWAS for introgression of desirable genes to advanced breeding populations for improvement of bunch and oil yield traits.

Next generation sequencing for oil palm improvement

The recent advances in genome sequencing through next generation sequencing (NGS) technologies provide opportunities to develop millions of novel markers, as well as the identification of agronomically important genes (Edwards and Batley, 2010). SNPs now dominate over other molecular marker applications, with the advancement in sequencing technology. Advancements in NGS enabled the development of high-density genetic maps. Genetic mapping places the markers in linkage groups based on their co-segregation. With the advancement of genomics technology, the generation of ESTs, genetic mapping and application of DNA chip technology have been employed in oil palm (Sambanthamurthi et al. 2009). A linkage map was constructed comprising 17 linkage groups with 117 RFLP loci, 384 AFLP markers and 23 SSR markers (Singh, 2005). Several QTLs for economic traits and the fruit colour genes (*vir*) have been successfully tagged in the linkage map. The markers associated with shell thickness have been identified. The ESTs also provided a platform for large-scale functional analysis of the genes using microarrays.

With the recent surge in next generation sequencing, the 1.8 Gb *E. guineensis* genome was sequenced with a combination of Roche/454 and Sanger Bacterial Artificial Chromosome (BAC) end sequencing (Singh et al. 2013b). In addition, transcriptome data from 30 tissues and a draft sequence of the South American oil palm, *Elaeis oleifera* were reported. A total of 34,802 genes were predicted, including oil biosynthesis genes, homologues of WRINKLED1 (WRI1), and other transcriptional regulators, which are highly expressed in the kernel (Singh et al. 2013b). In the subsequent studies, the gene responsible for the shell thickness (*SHELL*) was identified and mapped (Singh et al. 2013a), delivering the opportunity for further exploitation in breeding programmes. Recently, an SNP based high density linkage map was constructed using genotyping by sequencing approach, and 3 QTL affecting trunk height and a single QTL associated with fruit bunch weight were identified (Pootakham et al. 2015). The sequence information provides the opportunity to mine other key genes responsible for higher productivity and resistance to biotic and abiotic stress.

Omics technology

Omics technology promises consistency and predictability in plant breeding towards better yield and quality food crops. Several emerging omics technologies have been introduced to oil palm research for unravelling the molecular mechanisms of oil palm system biology under various conditions.

Multidisciplinary approaches such as genomics, transcriptomics, proteomics and metabolomics are being developed and adopted to pave this endeavour forward. The advancement of omics platforms has provided valuable resources for the discovery, assessment and establishment of molecular markers and precise gene modification through genetic engineering. The omics methodologies applied in oil palm research have facilitated extensive discoveries of indicative transcripts, proteins and metabolites associated with yield traits such as fruit ripeness, fruit quality, fruit form and lipid formation (Wong *et al*., 2017; Hassan *et al*., 2016; Ooi *et al*., 2015; Teh *et al*., 2014; 2013; Loei *et al*., 2013; Neoh *et al*., 2013; Dussert *et al*., 2013 and Hassan *et al*., 2014). The application of transcriptomics, proteomics and metabolomics has been employed to investigate the interactions between oil palm and *G. boninense* fungus (Nusaibah et al. 2016; Dzulkafli et al. 2016; Tee et al. 2013; Nurazah et al. 2013; Alizadeh et al. 2011 and Aswad et al. 2011).

Genome editing

In essence, the advances in genome sequences and genetic engineering techniques have also laid a foundation towards genome editing approach. Genome editing is a new technology that could be applied to allow the modification of oil palm genome in a precise and predictable manner without introducing foreign DNA. The use of genome editing in plant for improvement of various traits was reviewed by Malzahn *et al*. (2017). Genome editing involves the introduction of targeted DNA double-strand breaks (DSB) using engineered nuclease and stimulating DNA repair mechanisms. Zinc finger nuclease (ZFN) and transcription activator-like effector-based nucleases (TALEN) are sequence specific nucleases with DNA binding domain commonly used in genome editing for targeting DNA mutagenesis. A simpler genome editing approach known as clustered regularly interspaced palindromic repeats (CRISPR) has been developed, which allows more effective regulation of targeted gene. The CRISPR/Cas 9 system is an adaptive of bacterial II immune system which requires Cas 9 nuclease to degrade DNA that matches to a single guided RNA (sgRNA) (Malzahn et al. 2017 and Song et al. 2016).

CONCLUSION

Advancement in sequencing technologies has had a great impact on crop genetics, enabling the sequencing of genomes and transcriptomes of several crops. A massive re-sequencing and gene expression studies are essential to identify the key genes responsible for a desired trait and to find its allele variability. Utilization of this knowledge in crop breeding would empower the development of better crop varieties and may lead to a second green revolution. This would reduce the hunger of billions and revolutionize the economies of developing tropical countries. Through these post-genomic researches, the oil palm can be manipulated towards the production of highest possible yield with sustainable practices to fulfill the mission for advancing the palm oil industry.

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