Applications and challenges of biotechnology in oil palm breeding

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Abstract. Biotechnological applications in oil palm breeding comprises tissue culture propagation (TC), genetic modification (GM), marker-assisted breeding (MAB), and marker-assisted selection (MAS). Commercial success of oil palm TC has been hampered by the fruit mantling somaclonal variation risk, inefficient cloning process and inefficient ortet selection. With the imminent availability of the mantling marker and subsequently markers for cloning and palm selection efficiencies, the scenario would change. Despite early enthusiasm, GM oil palm remains at the R&D level and only for the high oleic trait, because of technical complexity and public sensitivity. Marker-assisted breeding, population genetic diversity, homozygosity determination and linkage disequilibrium have made most impact in oil palm breeding. With the revelation of the oil palm whole genome sequence, the feasibility of MAS for many major quantitative gene locus (QTL) traits e.g. shell, fruit colour, mantling, long stalk, lipase are being expedited. Meanwhile for minor QTL controlling yield and other traits, oil palm breeders are testing the feasibilities of genome-wide breeding/genetic value estimation for MAS of hybrid parents, families and individuals. Undoubtedly, biotechnological tools will continue improving but their success depends on the field breeder who provides the essential genetic trials to develop the biotechnological tools and to validate their utility.

1. Introduction

The oil palm is a relatively new crop with about a hundred years’ history [1]. With a generation cycle of about 8-10 years, it has undergone only about 10 cycles of scientific plant breeding. Nevertheless, through breeding cum agronomy research, yields of less than 1 t/ha oil in the wild palm groves have been improved to 7-9 t/ha achievable on a plantation scale to 18 t/ha potential yield. Oil palm breeding has also achieved 1-1.5% yield improvement per year comparable to those achieved by major crops [2].

The main objective of oil palm breeding since the early days has been high and precocious oil yield. This has resulted in the development of vigorous tall growing varieties that poses increasing manual harvesting problems in labour scarce plantation areas. The extensive and intensive (repeated) plantings of uniform narrow genetically based bred varieties responsive to high agronomic inputs and good growing
conditions have also led to their genetically vulnerability to existing and new pest and diseases (P&D): leaf-eating insect pest attacks e.g. leaf miners; root, stem and bud rots e.g. Ganoderma Basal Stem Rot, Fusarium Wilt, Lethal Bud Rot and to the acute environmental stresses (global warming, extreme temperature and rainfall fluctuations, storms) attributed to climate change arising from anthropogenic CO2 rise in the atmosphere. The oil palm has to adapt (via resistance/tolerance/resilience) to these stresses. It also has to mitigate against CO2 and greenhouse gas rise by being more efficient in its use of resource inputs e.g. N fertilizer, water, and light. Hence new breeding objectives and approaches are needed: breeding for resource use efficiency (RUE); breeding for resistance/tolerance/resilience to biotic (P&D) and abiotic (environmental) stress, and breeding for biodiversity.

Recognizing that the traditional breeding approach requires 20-40 years to produce a new variety (depending on the starting genetic base) will grossly be inadequate and slow to respond to the Industry needs to meet the rapidly changing economic and environmental challenges, oil palm breeders have turned to biotechnology as by other crop breeders.

2. Oil Palm Biotechnology

Biotechnology in oil palm breeding encompasses in vitro (tissue culture) propagation, genetic modification (GM) or transgenics, genome sequencing and molecular breeding (DNA marker-assisted breeding or MAB, and marker-assisted selection or MAS [3].

3. In Vitro Propagation

Oil palm tissue culture (OPTC) clonal propagation of elite palms was the first attempt into biotechnology [4, 5]. The rationale was that commercial oil palm hybrid progenies were genetically heterogeneous (arising from the use of non-fully inbred parents) with individual palms capable of yielding more than 30% of the mean. OPTC R&D began in the mid-1970s but only became a technology with available commercial plantlet sale in the new millennium [6]. However, to date clonal palms have yet to make a significant impact on field plantings constituting less than 2% of the oil palm seeds market and plantings [7]. The mantled fruit somaclonal variation risk [8] leading to palm sterility, cloning (calllogenesis, somatic embryogenesis) inefficiency resulting in high production cost, and lower than expected yield advantage of clonal palms have been the attributing factors [6, 9, 10]. Recloning and liquid cultures would reduce the second and third inefficiencies but with the attendant higher risk of fruit mantling. The lag time with recloning would also reduce its advantage over concurrently improved hybrids.

Commercial production has been achieved through cloning a basket of ortets or clonal parents, limiting production per culture line from the gel and liquid suspension systems and from primary and secondary (reclones) clones to hedge on mantling risk and yield advantage yet achieving commercial production levels [6]. Packaging a mix of clones is also done in the field to hedge against mantling, extended juvenility, poor pollination, P&D and GxE (genotype x environment interaction) risks with the consequence of compromising on the maximum yield achievable with clones.

The molecular bases of fruit mantling and calllogenesis cum somatic embryogenesis in oil palm clones have been actively researched [11, 12, 13]. Fruit mantling is the consequence of hypomethylation of the Karma transposon in the homeotic genes [14]. Histone modification may also be involved. Some prospective molecular markers for calllogenesis and somatic embryogenesis are thus anxiously anticipated. When available, clonal propagation would expedite the production and planting of clonal palms not only for high oil yield but also for ease of harvesting, value addition traits (oil quality, vitamins, phytonutrients), P&D and environmental stress resistance/adaptation traits and also the early exploitation of new genetic material to reduce genetic vulnerability and enhance sustainability of the plantation [15].
4. Genetic Modification

Genetically modified (GM) oil palm has been researched since the 1990’s. The primary traits targeted were high oleic acid for healthy low unsaturated oil [18, 19] and high PHBV (polyhydroxy butyrate valeate) for bioplastics production [18]. Such putative transformed palms have been obtained via biolistics and Agrobacterium-mediated methods [19, 20, 21, 22, 23] and are being validated for stable single gene copy incorporation and regeneration in the laboratory and biosafety containment houses. In common with the world trend, GM oil palm work has been downsized to just the oleic R&D program as proof of concept due to technical difficulties (multiple biochemical pathways, tissue-specific promotors and genes involved in many of the traits [24], cumbersome and biosafety test requirements and public sensitivity. Recently, the gene editing technique which allows targeted in situ DNA alteration without or transient use of transgenes has been touted as a solution to the controversy [25]. If so, GM oil palm research will resume its importance [24] especially with the revelation of the oil palm whole genome and some key trait sequence [26, 27, 28].

5. Marker-Assisted Breeding

5.1. DNA marker or molecular marker-assisted breeding (MAB)

MAB has made most impact in oil palm breeding: genotype authentication of breeding parents and hybrids, clonal ortets and ramets is now standard practice [29]. Besides facilitating accuracy and precision of breeding and cloning, it has application in plant variety protection (PVP) although currently it plays only as supporting evidence to phenotypic discrimination [30]. Population molecular diversity studies for breeding heterotic population organization, germplasm prospection and core collection which facilitate the selection, maintenance and exploitation of key genotypes and populations have also now become standard practice in many oil palm breeding programs [31, 32, 33, 34]. Related to these are also homozygosity testing which expedites development of inbred (also isogenic and recombinant inbred lines) via single seed descent or doubled haploidy [15, 35].

5.2. Genetic mapping, linkage studies and gene discovery

These serve as prelude to developing markers for key genes besides understanding their organization and function. This active area of research has culminated in the elucidation of three genome sequence maps for E. guineensis (MPOB, ACGT, Sime Darby) and one for E. oleifera by MPOB [26]. This has facilitated the discoveries of the genomic bases of the shell, virescens, and fruit mantling traits [14, 27, 28]. It is now apparent the oil palm genome varies with the different populations and likewise the genes controlling the traits e.g. shell, virescens with five separate mutations each found to date. As such, different or a suit of markers may need to be deployed in marker-assisted selection (MAS).

6. Marker-Assisted Selection

Being an outbred perennial tree crop, the oil palm lacks traditional genetic maps for phenotypic traits. With the progressive advent of fine or more precise DNA markers e.g. RFLP (randomized fragment length polymorphism, Amplified FLP, SSR (simple sequence repeats), SNP (single nucleotide polymorphism), culminating in the publication of the oil palm whole genome, and together with the reduced cost of high-throughput re-sequencing provided a better opportunity for high density precise mapping of important traits [19, 36].

MAS is currently actively pursued in oil palm breeding. MAS allows early and efficient selection saving time, space, effort and consequently money. Precise markers for the shell and virescens fruit traits are available; the former useful in separating out the tenera (T) palms for their dedicated evaluation from the complicating co-segregant dura (D) and pisifera (P) palms. The D palms are usually of little interest in
such trials, while the Ps are of interest in DxP progeny-testing cum pollen source for seed production and preferably planted as a separate “pollen garden”. The inclusion of the vigorous growing female sterile P segregants in various proportions in the trial TxT plots will also bias the agronomic evaluation of the T palms and plots. The shell marker is also useful in assuring the legitimacy of commercial pedigreed DxP seeds [37] although pedigree DNA fingerprinting using SNP/SSR would be better. Markers for other traits e.g. ease of harvesting (dwarf, long stalk, virescence, delayed fruit shedding, low lipase, high callogenesis/embryogenesis are currently being sought and developed [23, 38, 39].

Traditional MAS deals with simple traits controlled by one or few major genes and the backcross breeding method is the most common approach and in biparental crosses. Quantitative traits e.g. yield, disease resistance, are controlled by quantitative trait loci (QTL) which may be a few with major effects, many with minor effects with or a combination of both and may be spread all over the genome rather than confined to certain chromosomes. This is particularly important for a population improvement (recurrent selection) breeding program.

Unlike linkage analysis, GWAS (genome-wide association studies) provides access to the total meiotic recombination accumulated in a large population during evolution to increase the mapping resolution. The GWAS-GEBV (genetic expected breeding value) uses thousands of SNPs to associate with the individual phenotypic differences in an essentially multiple regression approach. Only major QTL i.e. significant SNP-trait associations are used and may lose out on minor QTL with this approach [40]. Sub-population structure and kinship effects have to be removed. Significance testing level also needs to be very stringent ($\alpha = 10^{-5}$) to avoid false positive results. The method also focuses on common variation, many of the associated variants are not causal.

The GS-BLUP (genomic selection best linear unbiased prediction) or mixed model approach uses all the markers irrespective of statistical significance and is more favoured by plant and animal breeders currently [41, 42]. As in GWAS, a large population of individuals are genotyped cum phenotyped. All marker and phenotypic data are fitted into a single statistical (mixed) model, called model training. The model is then used to predict the breeding values of ‘future’ individuals that have genotyped but not phenotyped.

Applications of GWAS and GS in oil palm both for parent breeding value (BV) and hybrid family and individual palm genotypic value (GV) predictions are currently being field validated e.g AAR. Sime Darby [43, 44]. If proven successful, much breeding time and effort could be saved and expected breeding progress achieved. The BV approach being based on family mean data would likely to be the more useful approach. Individual plant data and selection is prone to environmental and genotype x environment interaction effect complications. Sample size, population structure and kinship issues would also need to be accounted for to obtain valid predictions. The GS approach also allows the incorporation of ‘omics’ (proteomics, metabolomics, phenomics) data which would more pertinent in breeding for disease resistance (host-parasite interaction) and stress adaptation (genotype x environment interaction) traits [45, 46, 47, 48].

7. Conclusions

Biotechnological tools and molecular breeding are here to stay if not indispensable in oil palm breeding. Oil palm breeding despite its long generation cycle disadvantage has been keeping abreast of the cross-cutting biotechnological advances from other annual and perennial crops. There will always be challenges and bottlenecks which could be solved or circumvented through further R&D. While undoubtedly biotechnological tools will keep on improving, the ultimate success of their application lies in developing a set of good field trials to provide reliable data which allows the continuous development of new biotechnological tools and the validation of their applications in breeding improvement and these are under the purview of the field plant breeder!
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