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GENOMICS AND PLANT BREEDING

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ABSTRACT

The recent announcements of the breakthroughs in obtaining the oil palm genome sequence map herald a new chapter in oil palm genetic improvement. These breakthroughs will spur the further development of oil palm genomics. This article examines how genomics would impact plant and oil palm breeding.

The knowledge derived from genomics research in terms of the DNA structure of a gene, how it functions and interacts with other genes to produce a trait, its homology and synteny of genes across species, and its derived tools, as well as linkage maps, gene discovery (candidate genes), and efficient markers, would allow new genes or alleles to be discovered and transformed into breeding populations to broaden their genetic base for further breeding. Marker-assisted selection (MAS) saves effort, time and space, and can be more efficient than field phenotyping. Cultivars from MAS for monogenic traits are available for a number of crops. MAS for quantitative traits has still to contend with quantitative trait loci (QTL) x environment interaction, QTL x host interaction, linkage, epistasis, inaccurate phenotyping and false positive linkage issues. Genetically modified (GM) cultivars are becoming more available with decreasing biosafety concerns and public misperceptions.

The application of genomic knowledge and tools in oil palm breeding is hampered by the crop's long generation cycle, large space requirement for field testing, and consequently small population sizes and paucity of diverse uniform experimental lines to develop and validate the tools. Hope lies in the use of model species to expedite this. MPOB has developed a number of putative transgenics, trait-linked markers and QTL, but what is needed is for the private industry to validate them with their own genetic materials and their forte to translate them into cultivars. With the rapid pace of development in genomic science and technology and the increasing number of plantation companies having genomics capability, good collaborative efforts and strategic partnerships to develop these genomic tools for the plant breeder to derive superior cultivars cost-effectively and readily cannot be over-emphasised.

Keywords: genomics, breeding application, oil palm.

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INTRODUCTION

The recent media announcements of breakthroughs in the complete sequencing of the oil palm genome by two private plantation companies officially heralded a new chapter in the history of oil palm genetic improvement. The oil palm can take its place in the world as another major crop that has had its whole genome map sequenced (at least in its draft form).

What is the consequence of this revelation of the oil palm genome?

Claims of quantum leaps in yield and other trait improvements have been made with the prospective technologies ensuing from these breakthroughs. My role today is to give a broad overview of how genomics will impact plant and oil palm breeding from a field breeder's perspective (as opposed to the molecular breeder who manipulates genes and traits at the laboratory level), using experiences from other crops as gleaned from recent literature.

GENOMIC OUTCOMES

Presumably with the revelation of the genome sequence map and its final annotation and reconciliation, the following will be the eventual

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expected outcomes (Griffiths *et al*., 2008):

Scientific Knowledge

- The DNA structure and arrangement of the structural genes and their regulatory genes of some, if not of all, the important traits, *i.e.* genomics.
- Understanding how the DNA of the genes (for specific traits) are transcribed into mRNA (transcriptomics), translated into proteins (proteomics), assembled in the metabolic pathways (metabolomics) and the final external expression of the trait (phenomics), *i.e.* functional genomics (*Figure 1*).
- Comparison of genome structure and sequence across (related/unrelated) species, *i.e.* comparative genomics, which is perhaps the most useful for studying the genomes of crops.

Perhaps of particular interest to the plant breeder are the genomic/molecular tools and products that would result.

Genomic Tools/Products

Linkage maps (Figure 2). Through reconciliation of the physical and genetic maps, the location and order arrangement of the genes and their regulatory sequences on the different chromosomes can be ascertained.

Gene discovery. Through an understanding of how genes work in contributing to traits, new alleles of the same trait, different sources of genes for the same or different traits, and also regulatory genes perhaps lost through the course of evolution, and natural or artificial (breeding) selection can be uncovered through:

- functional genomics and reverse genetics;
- comparative genomics – examining comparative sequence homology and synteny (similar gene order in blocks of genes) across species; and
- candidate gene analysis – the use of known genes for important traits in model species (*e.g. Arabidopsis*, rice) to search for similar genes in different crop species.

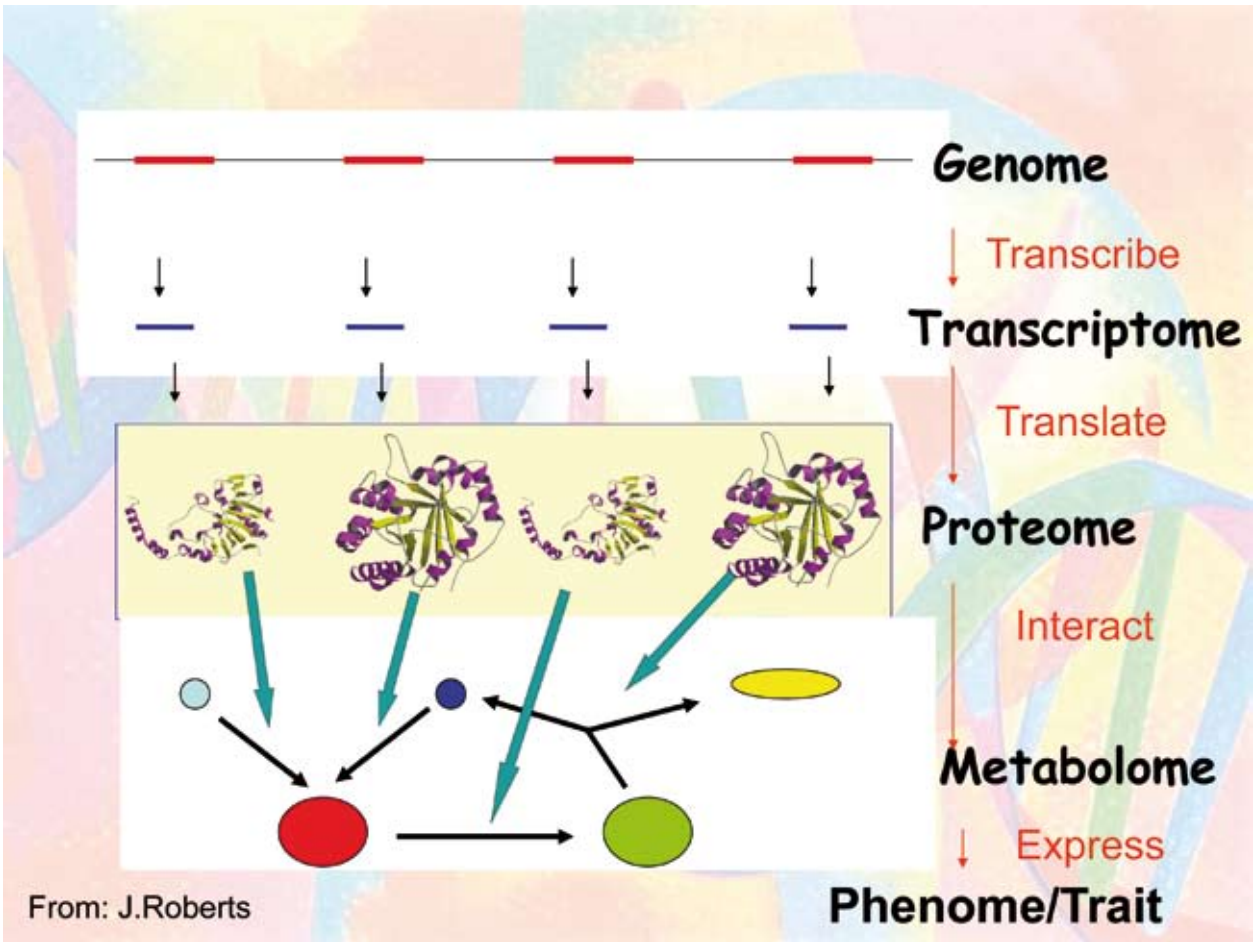

Figure 1. Functional genomics.

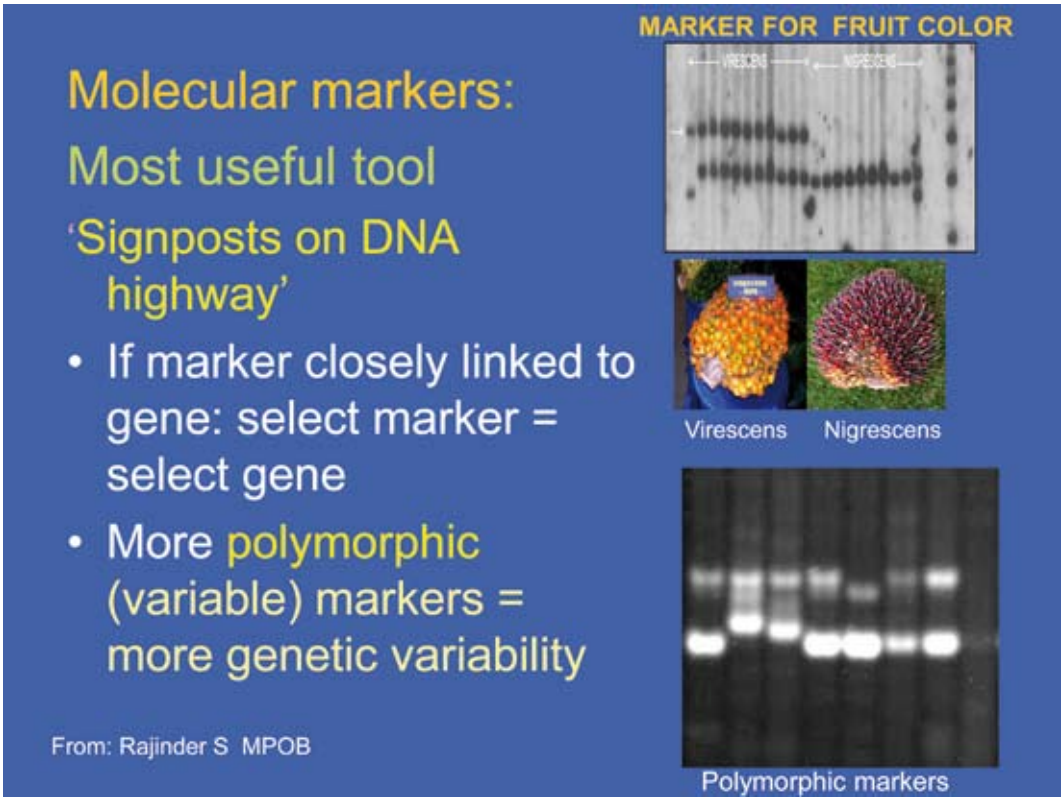

Figure 2. Molecular markers.

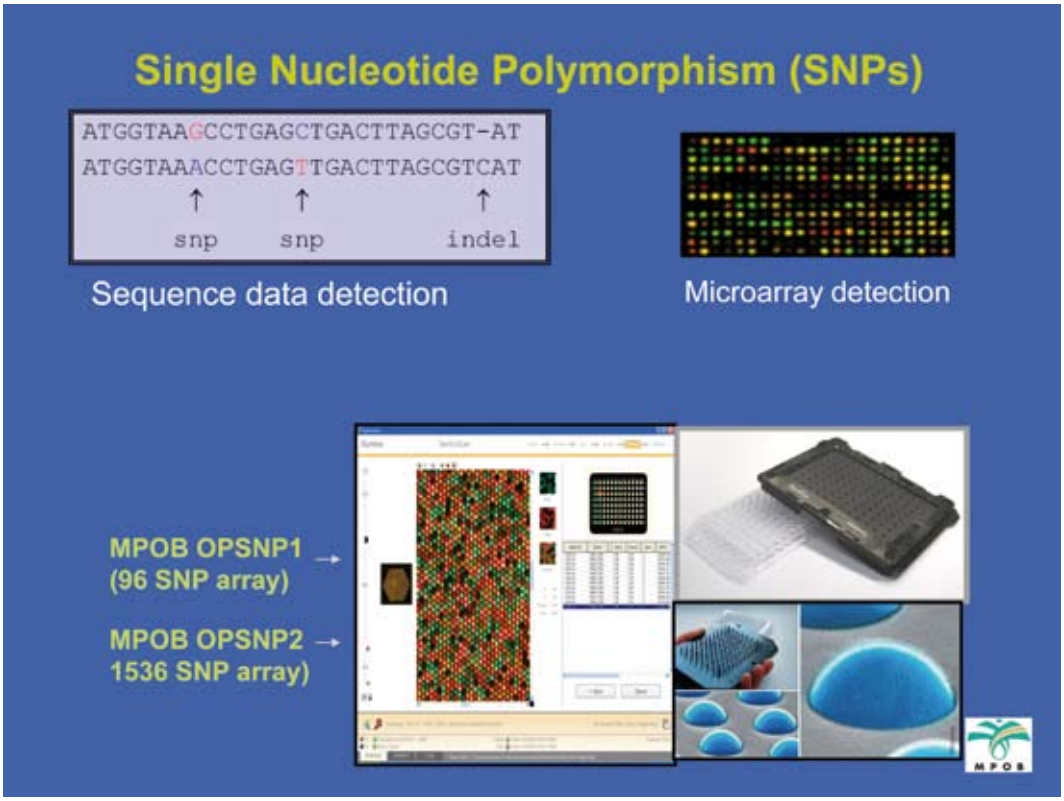

Figure 3. SNPs and microarrays.

These could then be screened for in natural populations, or even reverse-engineered through genetic modification (GM) approaches.

Efficient markers. Instead of searching for closely linked markers to the desired gene to minimize recombination through, for example, flanking markers, interval and fine mapping, tightly linked markers next to the gene can be constructed (*Figure 3*).

Markers can also be obtained via the candidate genes approach. The gene itself or, better still, an SNP (single nucleotide polymorphism) marker within the gene is perhaps the ideal or 'perfect' marker as recombination can be ruled out, and especially if it gives the mutant type directly (Collard *et al*., 2005, Mayes *et al*., 2008).

Efficient high through-put marker technology continues to improve, *e.g*. Affymetrix and DArT. The SNP microarrays or chips capable of analysing simultaneously 100 000 loci or perhaps all known genes which are responsible for every conceivable trait are now available (Meaburn *et al*., 2006) (*Figure 4*).

The host of available genomic techniques/ tools/technologies and their continuous development and improvement can be mind-boggling! The important question is how will this impact on plant breeding and on how the field breeder does his work?

WHAT IS PLANT BREEDING?

Plant breeding would best be explained by its definition, principles and procedures.

Definition

A 'lofty' definition for plant breeding would be: the application of genetic principles in manipulating plants by hybridisation and selection to improve cultivars suited to specific environments and production practices, and to provide food, feed, fibre (and also fuel and drugs) for the betterment of mankind.

Principles of Plant Breeding

The principles of plant breeding involve the setting up of breeding strategies/objectives of a breeding programme, followed by implementation of the actual breeding plan/programme/ procedure decided upon.

Breeding strategies/objectives. The breeding strategies/objectives are derived from the following knowledge:

- the growing environment of the crop, *i.e.* agronomy;

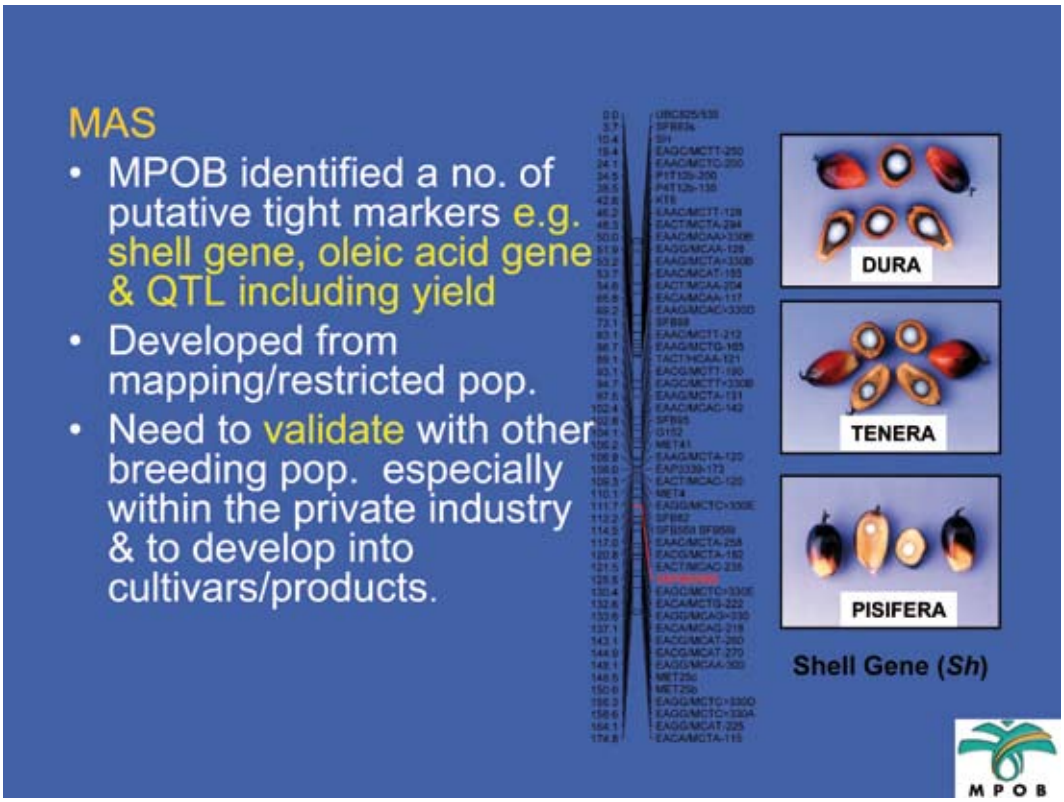

Figure 4. Marker-assisted selection.

- the biology of the crop, *i.e.* botany, genetics, physiology, biotic (pest and disease) and abiotic (physical, mineral) stresses, product quality attributes (nutrients, chemical composition);
- field experimentation, *i.e.* applied statistics and field trial management; and
- clientele – community/producer, end-user, *i.e.* marketing and business management.

A plant breeder thus not only needs to have a good knowledge of plant genetics (qualitative, quantitative, molecular) and the relevant biological and statistical sciences, but also in the case of a commercial plant breeder needs to develop some marketing and business management skills.

Plant Breeding Process

The plant breeding process typically involves the following stages:

- definition of objectives/goals: based on cultivar type (*e.g.* open-pollinated, single cross/mixed hybrids, clones). This is usually dictated or influenced by the crop's breeding/mating system and market needs;
- decision on the appropriate breeding method (*e.g.* pedigree/inbred variety, recurrent selection/hybrid variety, clonal breeding). This is also influenced by the breeding system and market;
- generation of genetic variability. This may be pre-existing, *e.g.* landraces, or generated through introgression or open-pollinated population/recurrent selected population from inter-mated parents;
- parent selection and hybridisation;
- selection of desirable variant genotypes from field tests;
- fixing and stabilisation of desirable genotype/s; and
- multiplication and marketing of the cultivar.

Breeding Methods

There are five main breeding methods related to the breeding and propagation systems of a crop (Allard, 1960).

Backross breeding (BC) method. This method is used to incorporate a desirable (donor) gene from a less improved (donor) variety into a recurrent advanced cultivar/breeding parent. Selection is simultaneously for the donor trait and the recurrent host genotype at each BC generation in the field. At least five backcrosses (BC5) are usually needed to develop a new cultivar.

Pedigree breeding method (PB)/Gene pyramiding (GP). This method is used in self-pollinated or inbred crops (*e.g.* wheat, rice, tomato and bean) to produce inbred varieties. The method may involve two or more parents with complementary traits, *i.e.* gene pyramiding. Selection usually begins in the F_2 or F_3 generation. Commercial inbred varieties are released at the F_8-F_{10} stages.

Hybrid breeding method (HB). This method has traditionally been used in cross-pollinated or outbred crops (*e.g.* maize, rape and oil palm) in place of mass selection to exploit hybrid vigour. Hybrid breeding has now been extended to self-pollinated crops with the availability of male sterile lines. Development of the inbred parents follows the pedigree method. By the F_5 stage, parent selection is based on progeny test performance to pick up heterotic combinations. Commercial hybrid seeds are produced from the F_8-F_{10} parents.

Clonal breeding method (CB). This method is usually practised in perennial tree crops which are highly outbred and possess long generation cycles, *e.g.* rubber and fruit trees. Selection begins in the widely segregating F_1 or F_2 of a cross of two open-pollinated (highly heterozygous) parents. Cycles of cloning, field testing and selection are practised until at least the fifth cycle (C5) before consideration for release as commercial clone cultivars.

Recurrent selection method (RS). This is a population improvement method practised commonly in cross-pollinated crops, *e.g.* maize, sorghum and oil palm. A number of selected parents are inter-mated. Parents are selected (with or without progeny-testing) from the segregating population from each generation of inter-mated parents. The aim is to sequentially accumulate favourable quantitative trait genes. Parents can be subsequently drawn out to be developed into inbred parents for hybrid production. This can also be achieved using the single seed descent method adopted in self-pollinated crops.

As commonly practised in all these breeding methods, selection and fixing of the monogenic or highly heritable traits occur in the early generations. Selection for quantitative traits is postponed till later generations when there is sufficient availability of uniform genotype lines for sequential field tests in statistically designed and replicated trials for evaluation of yield and other agronomic traits.

It takes from six to 12 years to produce a new cultivar for annual crops, *e.g.* wheat, rice and soyabean. However, in active breeding organisations with many overlapping programmes, new cultivars may be released every new season. In contrast, in oil palm it takes at least 30 years to

develop a reasonably uniform cultivar from the initial recombinant cross, and usually not less than 20 years even with overlapping programmes.

Evidently plant breeders have been constantly seeking means to short circuit this tedious process since the dawn of the science of modern plant breeding (Allard, 1960), although impressive gains have been made using the classical approaches. Examples of these successes include modern hybrid maize from the early dent corn, dwarfing genes and the Green Revolution, and modern oil palm varieties yielding close to 10 t oil per hectares compared to less than 500 kg from the wild palms.

APPLICATION OF GENOMIC TOOLS IN THE PLANT BREEDING PROCESS

Creation of Genetic Variability

Through the use of molecular markers and their availability in high through-put microarrays *e.g.* Affymetrix and DArT, in revealing genetic polymorphisms and their diversities, breeding and germplasm populations can be organised into divergent or heterotic groups to facilitate intra- and inter-population improvement (Bernardo, 2002; Mayes *et al.*, 2008).

New alleles or genes for the same trait, or different genes from near relatives or unrelated species and also regulatory genes obtained through gene discovery techniques (functional genomics/synteny/candidate genes) can be introgressed into the breeding populations via conventional hybridisation or transformation/GM including position cloning techniques (Tanksley and McCouch, 1997).

Some of the technical issues in efficient genetic transformation, besides being limited to monogenic traits, include unstable incorporation, partial or multiple copies of the transgene and gene silencing, all of which requiring much field testing (Murphy, 2004). Such issues are gradually being resolved or ameliorated with new technological improvements.

Selection of Desirable Genotypes

Due to the tedium and difficulty in accurately phenotyping plants, requiring much time, effort and space, for their subsequent selection, breeders have always sought for aids or markers to facilitate this.

Morphological/biochemical markers are limited or inefficient, and the discovery of abundant molecular markers has spurred the development of this useful tool. Molecular marker-assisted selection (MAS) has been most successfully applied in the breeding of monogenic traits (*Figure 5*). This is achieved through the backcross breeding (BC) and pedigree (for single gene) and gene pyramiding (for multiple genes) methods.

Figure 5. Oil palm genetic map.

In marker-assisted BC (MAB) or marker-assisted introgression (MAI) breeding, in the BC generations, simultaneous selection for the designated marker for the donor gene and a sample of random markers representative of the recurrent parent host genotype is practised. Two BCs are considered sufficient to accomplish the programme's objective instead of at least five BCs with the conventional method (Collard *et al*., 2005).

In the PB/GP method, incorporation of the desired qualitative trait/gene is by selfing and selection to fix the marker, and thus the linked gene, at the homozygous state in the early cycles of selection. Quantitative traits, *e.g*. yield, are selected after field testing in replicated trials. There is a little saving in cultivar development time, only in early and efficient selection of the qualitative trait, and some space saving by not having to field plant the non-marked genotypes. In GP, MAS is practiced simultaneously on more than one trait/allele, *e.g.* disease resistance for more than one pathogenic race (Zhang *et al*., 2006).

Quantitative Trait Loci (QTL)

QTL are genes controlling quantitative traits. They are spread over the genome (chromosome-wide), and may be concentrated in some regions as blocks. QTL are revealed (and mapped) using markers within segregating populations for the quantitative trait by various methods, *e.g.* single marker analysis with linear regression (*e.g*. using QGene and MapManager QTX software), simple and composite interval mapping (*e.g*. MapManager QTX and QTL Cartographer), bulk segregation analysis and selective genotyping. The contribution of QTL in explaining the variation in the quantitative trait can also be estimated (Collard *et al*., 2005).

There are traits with a few major QTL (accounting for >10% of effects) and some minor QTL (accounting for <10% of effects), *e.g.* disease resistance. The breeding methods to exploit major QTL are the same as for monogenic traits, *i.e*. using BC and GP.

Many traits of economic importance are controlled by many QTL with small effects, *e.g.* yield, oil content, protein content and drought tolerance, and are usually exploited by recurrent selection to accumulate the desirable loci via their associated markers. Variants of the recurrent selection method using markers (Bernardo and Yu, 2007; Wong and Bernardo, 2008) are:

- marker-based selection – selection solely based on markers;
- marker-assisted recurrent selection (MARS) – recurrent selection with subset QTL having significant contributions; and
- genome-wide marker-assisted recurrent selection (GMARS) – using a large random set of genome-wide markers.

Breeding programmes may use a combination of the variants, and may also include phenotypic selection.

The molecular recurrent selection method also saves time, space and effort by obviating certain intervening cycles of field testing.

Merits of MAS (Hospital, 2009; Xu and Crouch, 2009).

- Efficient selection – for traits with low heritability, which are difficult to measure, or are affected by the environment.
- Early selection – selection can be carried out before plants mature or at a stage when the trait is observable. This reduces the need for costly extensive nursery and field tests, *e.g*. for stress resistance.
- Reduction of effort – reduction in the need for cumbersome field trials for unreliable field phenotyping; only candidates having the desired markers (also homozygotes for the desired donor trait) are saved for further breeding and field testing.
- Avoiding the transfer of undesirable/ deleterious genes due to linkage drag, especially in introgression programmes with genes from wild species.
- Testing for specific traits where phenotyping is not feasible, *e.g*. due to quarantine restrictions.
- Shorter cultivar development time – substituting complex and time-consuming field trials with molecular tests.

Current limitations of MAS (Hospital, 2009; Xu and Crouch, 2009).

- GP for major genes or QTL may not work in multiple parent host genetic backgrounds. Likewise, the candidate genes approach may not work in a different host species.
- GP for major genes or QTL may not confer stable genotypes, *e.g.* for disease resistance.
- QTL vary with different environments (such as season, location and management) and host genetic backgrounds.
- QTL are also affected by GxE (genotype x environment) interactions, linkage/ pleiotropy and epistasis (gene interactions). In a complex polygenic system, these are inevitable, especially at the molecular level. Proposed approaches to circumvent GxE (similar to classical approaches) are by

clustering target environments into mega environments, and to seek for proprietary QTL or to seek for across population or environment QTL.

- QTL from a mapping population may not work in a breeding population. Mapping populations are usually derived from wide crosses with a mixed genetic background as compared to those in breeding populations.
- There may be a need to develop QTL for each breeding population and cycle. Different breeding populations will have different genetic backgrounds and breeding histories. The QTL effects would change with succeeding cycles and would need to be re-estimated, *e.g*. by the 'mapping as you go' approach (MAYG).
- Multiple trait selection (ca. 20-50) is commonly practiced in breeding and a selection index approach incorporating markers is being researched.
- Many false positive marker-linked QTL result from analytical and statistical deficiencies, especially when working with small plant populations (Bernardo, 2004).
- There is a lack of good field data (quantity and quality). This is perhaps the most important issue. Specifically designed crosses and experiments can be planted. This is a tedious effort, with results taking time to be obtained, and even then their accuracy cannot be fully assured, more so for a perennial tree crop. In oil palm, the coefficients of variation (CV) for yield in most progeny trials exceed 10% of the trial mean. As such, it is difficult to detect true differences of less than 15% (Soh *et al*., 1990). Illegitimacy and human error further confound the issue.

COMMERCIAL APPLICATIONS/ TECHNOLOGIES

A successful new technique results in a publication. A technique becomes a technology when a commercial product becomes available.

GM Crops

Currently, there are 30 GM cultivars in cultivation in the world, and the number is expected to increase to more than 120 by 2015 (cotton from 12 to 27, maize 9 to 24, rapeseed 4 to 8, rice 0 to 15, potato 0 to 8, and minor crops from 7 to 23), especially now that the European Union has relaxed its opposition to GM crops (JRC European Commission NewsRelease, 2009). Most of the suppliers of GM cultivars are private technology companies in USA and EU, but by 2015, GM crops from national programmes in Asia and Latin America are expected to be available although more for domestic markets. GM crops with new traits, *e.g*. improved oil and starch contents, nutrient composition and drought tolerance, besides current insect and herbicide resistance, would also make the scene by then.

MAS Crops

There are a number of MAS (MAB/MAI) programmes but these are seldom reported in published literature. The first MAS cultivar was released by Monsanto in 2006, and such cultivars would represent 12% or more of the commercial crops by 2010. There are a number of large MAS (MAB/MAI for pest and disease resistance, bread/ pasta making and cooking quality) programmes from public breeding programmes, *e.g*. wheat at CIMMYT, Australia and the US MAS Wheat Consortium. A few varieties have been released, *e.g.* Cadet and Jacinto rice with better cooking and processing qualities in US, Angke and Conde rice with bacteria blight resistance in Indonesia, USPT-ANT-1 anthracnose resistant line of pinto bean in US, and India's new downy mildew resistant pearl millet hybrid HHB67-2 line (Xu and Crouch, 2009).

Cultivars from MAS-QTL breeding are expected to make the scene only in a couple of years' time despite the large number of publications on this topic to date.

ISSUES IN COMMERCIALISATION

Transgenics (Murphy, 2004)

The greatest issues in the commercialisation of GM crops are biosafety tests and public acceptance. The biosafety test regulatory requirements to register a GM cultivar are very stringent and time-consuming, and hence the tests are very expensive. Also, there are only a few countries (*e.g.* USA, Spain) with acceptable credibility that can host biosafety testing of GM crops.

The general public in many countries, *e.g*. EU, Australia and New Zealand, has a negative perception of GM crops, largely due to sensitisation from anti-GM NGO (non- government organisation) activists. A large budget for public relation exercises and education is needed to promote a GM cultivar, adding to its cost of development. Fortunately, the general situation on both issues appears to be improving.

MAS (Dreher *et al*., 2000; Holiday, 2009; Xu and Crouch 2009)

The issues confronting the adoption of MAS technology for developing commercial cultivars are:

high investment cost to start-up; need for hardware, software and people-ware; expensive field trials; high through-put systems; reliable sample and data-tracking systems; and bioinformatics and decision-support systems.

The high cost of start-up for a molecular laboratory and investments in equipment, software and capacity building are not an issue for national laboratories as MAS is in their mandate. It would be the same for large private breeding companies if there are profits (or reduced costs) to be made, and especially with tax relief incentives. Technology keeps improving with high through-put systems, and the cost per sample analysed keeps decreasing. To circumvent the setting up of expensive and time-consuming field trials, existing field trial data can be exploited although analytical and statistical issues need to be resolved.

CONCLUSION

The foregoing discussions presume that all the relevant oil palm genomic science knowledge and tools would be available easily and soon. Nothing can be further from the truth as oil palm, being a perennial outbred tree crop, lacks good experimental populations (*e.g.* recombinant inbred/near isogenic/dihaploid/mutant/tranformed lines), or it is tedious to generate them, to facilitate genomics research and development (R&D). The reconciliation of the different draft genome maps into a coherent map would be a challenge and would take time as the maps were made with different approaches and on different genetic materials, and the probability of proprietary interest impeding collaboration exists. Mapping all the genes (even for only the desirable traits) on the oil palm genome map would be a long tedious effort as experienced in *Arabidopsis* which has a generation cycle time in terms of weeks compared to years in oil palm. The molecular genetic control in some of the plants' metabolic pathways is still unknown, or has turned out to be more complex than originally thought, *e.g.* for protein content and oil content. Model species, *e.g*. *Arabidopsis* and rice, can provide a model to better understand these physiological processes and their genetic control besides serving as 'surrogates' for testing the expression of oil palm transgenes.

Notwithstanding the above, in oil palm, MPOB is perhaps the most advanced in terms of its R&D and the development of prospective genomic tools, largely due to its early mandate and collaborative efforts with international centres of excellence in this area. In transgenics, MPOB has developed a host of putative transformants, *e.g.* high oleic and high PHB (polyhydroxy butyrate) bioplastics. These need to be subjected to the necessary laboratory tests to exclude partial and multiple copies of the transgene as most of the transformants were obtained via biolistics. They also need to be field-tested for stability in inheritance and expression of the trait, and for biosafety. Malaysia has gazetted its Biosafety Act, and MPOB has built its own biosafety facility. International accreditation and acceptability in this is crucial.

In MAS, MPOB has also developed a number of putative tight markers, *e.g*. shell gene and virescens gene, and identified QTL for a number of other traits including yield (Rajinder and Cheah, 2005). As these were developed from mapping populations or restricted breeding populations of small sizes, they need to be validated with other breeding populations available in the private industry.

Now that some private oil palm R&D companies backed by genomics giant companies or leading university laboratories have entered the game, smart partnerships/collaborative efforts should be made between MPOB and these companies (despite their different remits) to translate the findings into commercial products and cultivars.

In the larger context, research in genomic science and the development of the genomic tools for prospective commercial applications have led, and will continue to lead, to a better understanding of the physiological basis of the various desirable traits in the oil palm and its interacting environmental biota, *e.g*. pests and diseases, and soil microbes, and perhaps their eventual manipulation into cultivars.

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