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SureSawit™ *SHELL* - A Diagnostic Assay to Predict Fruit Forms of Oil Palm

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ABSTRACT

Genetic studies in oil palm have been impeded by its long breeding cycle and requirement for large tracts of land for field planting. The application of genomics tools provides an opportunity to overcome these constraints and move forward from the phenotype-driven research that had been the focus previously. In order to develop these tools, the Malaysian Palm Oil Board (MPOB) and its partners achieved a significant scientific breakthrough by decoding the E. guineensis *and* E. oleifera *genomes, in 2013. Obtaining high quality sequence assembly proved to be a valuable resource to identify genes linked to important agronomic traits of oil palm. In this respect, using a combination of classical genetics and whole genome sequence as a reference, the gene responsible for the three different fruit forms of* E. guineensis - SHELL - *was identified. The identification of the gene responsible for the fruit forms paved the way for the development of the SureSawit™* SHELL *diagnostic assay kit. The kit advances the application of molecular diagnostic tools in both oil palm breeding and commercial seed production.*

ABSTRAK

Kajian genetik sawit sering menghadapi halangan yang berpunca daripada kitar pembiakbakaan yang panjang dan keperluan kawasan tanah yang luas untuk penanaman. Aplikasi peralatan genomik membuka peluang untuk mengatasi halangan tersebut dan beralih daripada kajian berasaskan fenotip yang merupakan fokus sebelum ini. Bagi membangunkan peralatan genomik tersebut, MPOB dan beberapa rakan kongsinya telah mencapai kejayaan yang cemerlang dalam bidang sains melalui pengekodan genom E. guineensis *dan* E. oleifera *pada tahun 2013. Kehadiran himpunan jujukan genom yang berkualiti tinggi merupakan sumber yang bernilai untuk mengenal pasti gen yang berkaitan dengan ciri-ciri agronomi sawit yang penting. Sehubungan dengan itu, melalui gabungan maklumat daripada genetik klasik dan jujukan genom lengkap sebagai rujukan, gen yang bertanggungjawab terhadap pembentukan tiga jenis buah daripada spesis* E. guineensis *– SHELL – telah dikenal pasti. Penemuan gen yang berperanan dalam pembentukan jenis buah tersebut telah memberi laluan untuk pembangunan kit asai diagnostik SureSawit™* SHELL. *Penggunaan kit asai diagnostik ini akan menerajui penggunaan peralatan diagnostik molekul dalam bidang pembiakbakaan sawit dan penghasilan biji benih komersil.*

Keywords: oil palm, fruit form, shell gene, SureSawit™ *SHELL*, diagnostic assay.

INTRODUCTION

The oil palm industry is facing numerous challenges to improve productivity in a sustainable manner. Although widely accepted as the highest yielding vegetable oil crop per unit area, there is a continuous demand on oil palm to improve yields within existing acreage. The Malaysian Palm Oil Board (MPOB) adopted an integrated approach to help address the problem. It was for this reason that genomics research, especially efforts at sequencing the oil palm genome and identifying the shell gene was initiated at a modest scale over 10 years ago. However, improvements in sequencing technology and the advent of next generation sequencing (NGS) technology provided MPOB an opportunity to accelerate its genomics research in 2009. This led to MPOB and its consortium partners completing the genome sequence of both species of oil palm namely *Elaeis guineensis* and *Elaeis oleifera* in 2013. The deciphering of the genetic code of oil palm was published in the highly respected scientific journal *Nature* in July 2013 (Singh *et al*., 2013a), giving significant credibility to the scientific efforts of MPOB and Malaysia. The oil palm genome sequence is an important resource to the local and

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international scientific communities as well as those associated with the oil palm industry and other related plants. The availability of the genome sequence facilitated the discovery of the shell gene. This article describes the molecular diagnostic assay developed subsequent to the discovery of *SHELL* and its potential application in commercial seed production and improving breeding efficiency.

IDENTIFICATION OF THE *SHELL* GENE

The initial target after deciphering the oil palm genome was to identify *SHELL;* the gene responsible for the three known fruit forms of *E. guineensis*: *dura*, *pisifera* and *tenera*. The fruits of *dura* palm have a thick shell, while *pisifera* palms are shell-less and usually female sterile. The hybrid between *dura* and *pisifera* palms are known as *tenera*, which have a thinner shell. The thickness of the shell correlates to fruit size and oil yield. *Tenera* palms are more productive and the preferred commercial planting material as they result in 30% more oil per land area than *dura* palms (Corley and Lee, 1982). Researchers involved in the project had initially constructed a detailed genetic map to identify the location of *SHELL* in the oil palm genome. The genome build obtained from deciphering the oil palm genome was subsequently used to help pinpoint the exact gene responsible for the three different fruit forms. The mapping of the oil palm genome and of *SHELL* had been extremely challenging because of the large genome, long life cycle of the palm and difficulty of typing the widely-distributed commercial palm populations. The identification of *SHELL* was also published in the same issue of *Nature* (Singh *et al.*, 2013b). The publication of two back to back articles in the highly acclaimed scientific journal *Nature* was an unprecedented achievement for Malaysian scientists.

Although the monogenic, co-dominant inheritance of the three fruit forms was first revealed over 70 years ago, the gene responsible for the trait remained elusive, and its discovery was a subject of intense competition by many local and international research groups. The discovery of the gene was a proud achievement by MPOB, especially since the discovery also revealed the genetic basis for higher oil yields in *tenera* compared to *dura* and *pisifera*. The key to the discovery of the gene was MPOB's efforts in the last five decades to amass more than 100 000 wild oil palm plants. The investigators used this collection, along with carefully designed genetic crosses to identify *SHELL* and its two mutations. The discovery of *SHELL* has important implications in improving the efficiency of oil palm breeding and commercial seed production, paving the way for improving oil palm productivity. More importantly, MPOB owns all intellectual property rights to the shell gene, cementing MPOB'S position as a leader in technological innovations related to the oil palm industry worldwide.

DEVELOPMENT OF DIAGNOSTIC ASSAY FOR PREDICTING FRUIT FORMS

The discovery of *SHELL* allowed MPOB and its consortium partner, to develop the SureSawit™ *SHELL* diagnostic kit (*Figure 1*). The diagnostic kit allows all three fruit forms (*dura*, *tenera* and *pisifera*) to be distinguished in the nursery or even earlier, long before they are field planted (*Figure 2*). The SureSawit™ *SHELL* kit can predict for the three fruit forms with 100% accuracy. This has important implications in improving yield, especially among smallholders, apart from helping to produce new and improved varieties at a much faster pace through breeding. The development of the diagnostic assay, also for the first time, brings genomics to the forefront in improving oil palm productivity. The SureSawit™ *SHELL* diagnostic assay is a novel approach – similar to applying the blue ocean strategy to avoid planting of unwanted *dura* and *pisifera* seeds in commercial fields. Early detection of *dura*

Figure 1. The SureSawit™ SHELL *diagnostic kit reflects MPOB's commitment to improve productivity and sustainability of the country's oil palm industry.*

Figure 2. Samples at the nursery that can now be identified accurately as dura, tenera or pisifera *even before field planting and development of fruits.*

and *pisifera* during production of the hybrid *tenera* will prevent wastage in terms of planting and maintaining palms that give lower yield.

APPLICATION OF SureSawit™ *SHELL* DIAGNOSTIC KIT

The discovery of *SHELL* and the development of the SureSawit™ *SHELL* diagnostic kit by MPOB and its partner Orion BioSains Sdn Bhd is a classic example of genomics guided crop improvement. Seed producers and nursery operators can now apply the technology to ensure that only *tenera* palms are produced and planted in commercial fields (*Figure 3*). This is important as previously even with selective breeding about 10% of plants were reported to be the low-yielding *dura* form due to uncontrol wind and insect pollination, particularly in plantations without stringent quality control measures (Chin, 1995). Admittedly the level of *dura* contamination has reduced tremendously of late due to improved quality control during seed production; however, the problem still persists to some extent. The ability to ensure that all palms planted are the hybrid *tenera*, is a concerted effort towards good agricultural practice that will help improve the industry's overall productivity. The diagnostic kit is anticipated to become a routine requirement of the Codes of Practice for oil palm.

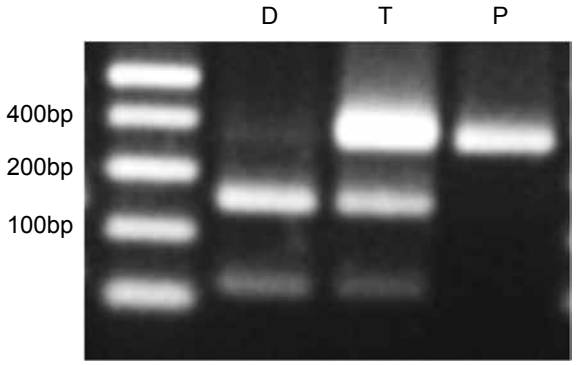

Figure 3. The different DNA profiles observed for dura *(D),* tenera *(T) dan* pisifera *(P) palms using the diagnostic assay applied on leaf samples of nursery plantlets.*

The discovery is important for smallholders in particular as they are often promised *tenera* plantlets, which more often than not are contaminated with *dura* plants, resulting in lower yields among smallholder plots. Reduced yield obtained from *dura* palms does to some extent contribute to the stagnating national average yields observed for the last 30-40 years. Prior to the discovery of the shell gene and the development of the diagnostic kit, it would take farmers up to six years to confirm that the palm planted is the desired fruit form, namely *tenera*. By this time, it is too late to remove the non-*tenera* palms, and farmers have to maintain the lower yielding non-desired palms for the next 20-25 years until the next re-planting cycle. The SureSawit™ *SHELL* diagnostic assay will also empower the enforcement team at MPOB to ensure that seed producers and nursery operators only produce and supply *tenera* planting material. A laboratory that provides a service to validate *tenera* planting materials at a reasonable cost for smallholders and other interested parties has also been successfully established.

The SureSawit™ *SHELL* kit will likely be most valuable to oil palm breeders in their efforts at developing and maintaining the male (*pisifera*) and female (*dura*) parental lines used for production of the commercial hybrid *tenera*. As the *pisifera* palms are female sterile, the only possible way to obtain the next generation *pisifera* palms is by making *tenera* x *tenera* or *tenera* x *pisifera* crosses. In these scenarios up to 25% of palms produced are the low-yielding *dura* form based on normal Mendelian inheritance. With the discovery of *SHELL*, seed producers can now use the gene itself via the SureSawit™ *SHELL* kit to distinguish the three fruit forms in the nursery long before they are field planted, thus enabling significantly enhanced breeding operations. In fact, for the first time, breeders will be able to segregate out *pisifera* palms and plant them separately at higher densities which encourage development of male inflorescence. Furthermore, *pisifera* palms in one location make it easier for breeders to manage and harvest pollen. Prior to the present invention, there was no known way to predict a palm's phenotype and breeding trials often comprised all three fruit forms, resulting in significant wastage of resources in maintaining palms of the undesired fruit forms. Similarly, *dura* maternal lines can be maintained in separate breeding plots for effective management and evaluation. The entire process of being able to separate out the male and female parental lines early enables a more focussed breeding programme that allows considerable savings in land, labour and resources applied in the programme. The discovery provides a paradigm shift in assisting oil palm breeders to reduce the breeding and selection cycle required to develop new and improved varieties. As the second largest producer of palm oil in the world, Malaysia has a responsibility to make such advancements that will improve the sustainability of oil palm agriculture, while improving yields for growers.

The discovery of *SHELL* and the development of the SureSawit™ *SHELL* diagnostic kit indicate a clear path towards more intensive use of already planted lands, and thus should lessen pressures to expand the land area devoted to oil palm, notably onto endangered rainforest - a major concern for

the environment and a rallying point for activists in recent years. The discovery of *SHELL* as well as the recent announcement on the identification of the fruit colour gene (Singh *et al*., 2014) have important implications in improving yield and is excellent news for the rainforest and its champions worldwide.

CONCLUSION

The genome mapping of the two oil palm species, discovery of *SHELL* and recent identification of the fruit colour gene paves the way for many more breakthroughs, and the oil palm genomes will continue to facilitate identification of genes responsible for other important traits such as yield, clonal abnormality, as well as quality traits such as disease resistance and height. The large germplasm collection and well executed breeding programmes are the assets that will assist MPOB to successfully translate the genome data into usefull applications that will impact yield of this important crop. The release of the oil palm genome data in the public database and dispensing of knowledge related to *SHELL* also fulfils MPOB's commitment to the oil palm industry. The purpose is to share its research findings, which opens the door for collaboration at both the national and international levels for the benefit of the industry.

REFERENCES

CHIN, C W (1995). Oil palm planting materials and quality control. *Proc. of the 1995 PORIM National Oil Palm Conference -Tech. in Plant*. p. 38-47.

CORLEY, R H V and LEE, C H (1992). The physiological bases for genetic improvement of oil palm in Malaysia. *Euphytica, 60*: 179-184.

SINGH, R; LOW, E-T L; OOI, L C-L; ONG-ABDULLAH, M; TING, N-C; JAYANTHI, N, RAJANAIDU, N; AMIRUDDIN, M D; ROSLI, R; ABDUL MANAF, M A; CHAN, K L; HALIM, M A; AZIZI, N; LAKEY, N; SMITH, S W; BUDIMAN, M A, HOGAN, M; BACHER, B; BRUNT, A V; WANG, C; ORDWAY, J M; SAMBANTHAMURTHI, R and MARTIENSSEN, R A (2013a). The oil palm SHELL gene controls oil yield and encodes a homologue of SEEDSTICK. *Nature, 500(7462)*: 340-344.

SINGH, R; ONG-ABDULLAH, M; LOW, E T L; ABDUL MANAF, M A; ROSLI, R; RAJANAIDU, N; OOI, L C-L; OOI, S E; CHAN, K L; HALIM, M A; AZIZI, N; JAYANTHI, N; BACHER, B; LAKEY, N; SMITH, S W; HE, D; HOGAN, M; BUDIMAN, MA; LEE, E K; DESALLE, R; KUDRNA, D; GOICOECHEA, J L; WING, R A; WILSON, R K; FULTON, R S; ORDWAY, J M; MARTIENSSEN, R A and SAMBANTHAMURTHI, R (2013b). Oil palm genome sequences reveals divergence of interfertile species in old and new worlds. *Nature, 500(7462)*: 335-339.

SINGH R; ENG-TI LESLIE LOW; LESLIE CHENG-LI OOI; MEILINA ONG-ABDULLAH; RAJANAIDU NOOKIAH; NGOOT-CHIN TING; MARHALIL MARJUNI; PEK-LAN CHAN; MAIZURA ITHNIN; MOHD ARIF ABDUL MANAF; JAYANTHI NAGAPPAN; KUANG-LIM CHAN; ROZANA ROSLI; MOHD AMIN HALIM; NORAZAH AZIZI; MUHAMMAD A BUDIMAN; NATHAN LAKEY; BLAIRE BACHER; ANDREW VAN BRUNT; CHUNYAN WANG; MICHAEL HOGAN; DONG HE; JILL D MACDONALD; STEVEN W SMITH; JARED M ORDWAY; ROBERT A MARTIENSSEN and RAVIGADEVI SAMBANTHAMURTHI (2014). The oil palm *VIRESCENS* gene controls fruit colour and encodes a R2R3-MYB**.** *Nature Communications 5, Article No. 4106*. DOI: 10.1038/ncomms5106.