PCR-BASED MARKER FOR DISTINGUISHING SKIN COLOUR OF OIL PALM FRUITS

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Oil palms can be distinguished based on the colour of their fruit coats. The two most common types are *nigrescens* (*Nig*) and *virescens* (*Vir*) (*Figure 1*). *Vir* fruits undergo a clear colour change on ripening, making it easier to identify the ripe bunches for harvesting. The fruits of the more commonly occurring *Nig* palms undergo only minimal change in colour upon ripening, thus, requiring loose fruits as an indicator of bunch ripeness. With the current labour shortage in the Malaysian plantation sector, the time spent on collection of loose fruits can have serious repercussions on productivity in the oil palm industry. In fact, as a result of labour shortage, one of the areas most neglected in harvesting is loose fruit collection (Hoong and Donough, 1998), which has been suggested to be one of the main reasons for the decline in oil extraction rate (OER) in Malaysia (Corley and Law, 2001). The commercial objective of reaching the best compromise between obtaining the maximum amount of oil in the bunch and having only a few loose fruits can be realized if the palms in the plantations are *virescens*.

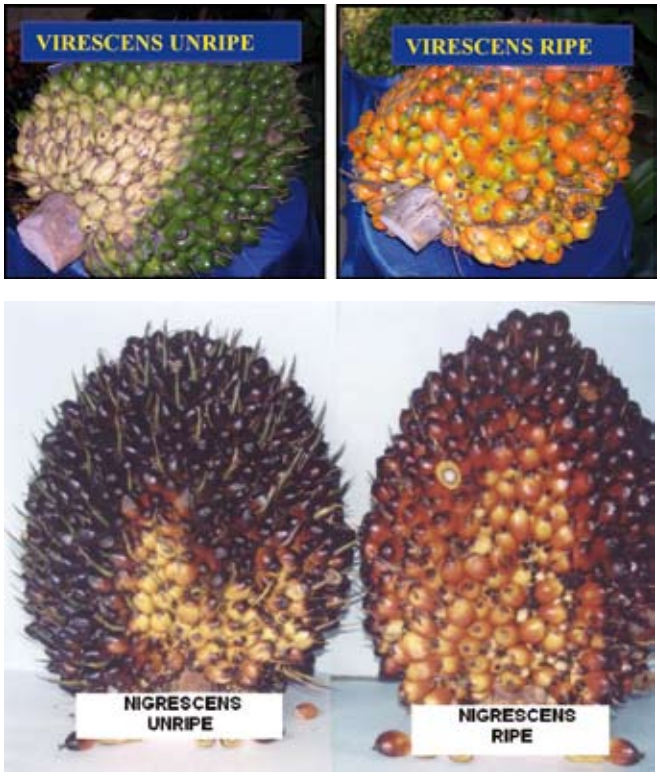

Figure 1. Comparison of virescens *and* nigrescens *bunches.*

The *Vir* trait is in fact monofactorial and dominant. As such, identification of its allele and introgression into non-abscising genotypes will also allow the identification of ripe bunches with reduced crop loss from fallen fruits, thereby improving yield.

IDENTIFICATION OF MARKERS LINKED TO THE FRUIT COLOUR GENE

The use of restriction fragment length polymorphic (RFLP) probes for differentiating the skin colour of oil palm fruits has been described by Rajinder *et al*. (2006). Although the process is effective, RFLP analysis is expensive, time-consuming and laborious.

To overcome these shortcomings, a microsatellite or simple sequence repeat (SSR) probe linked to the fruit colour trait was developed to differentiate the skin colour of oil palm fruits. The SSR marker was developed from the original sequence of the RFLP probe.

DETERMINING THE POSITION OF THE SSR MARKER LINKED TO THE FRUIT COLOUR GENE ON THE GENETIC MAP

The SSR marker linked to the fruit colour gene in oil palm (MET16-ssr) was located on a genetic map constructed using a population segregating for the trait (*Figure 2*). This marker is located close to the fruit colour locus (*Vir*). More importantly,

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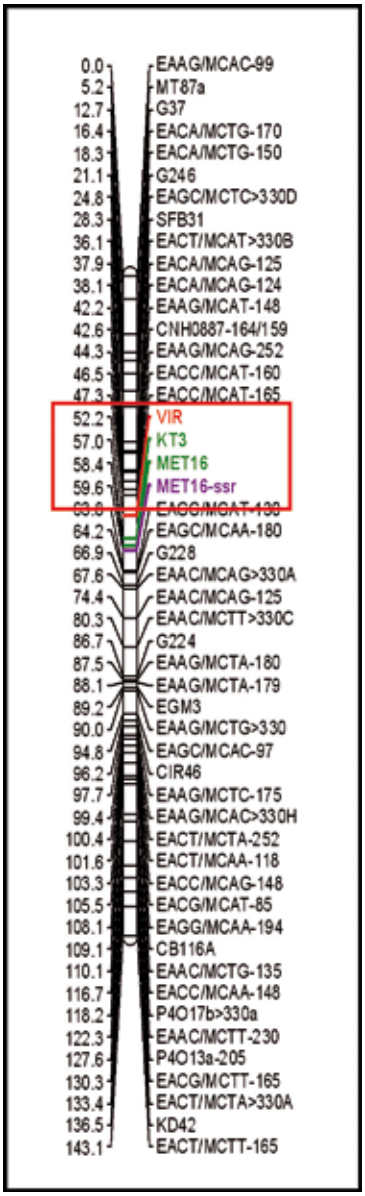

*Figure 2. Mapping the fruit colour gene (*Vir*).*

the MET16-ssr marker also mapped close to the probes MET16 and KT3, the two RFLP markers that were previously shown to be able to differentiate the skin colour of oil palm fruits. The SSR marker MET16-ssr also showed a co-dominant profile, similar to that of the RFLP probes reported previously.

TESTING THE SSR MARKER LINKED TO THE FRUIT COLOUR LOCUS

Figure 3 displays a subset of the mapping population analysed with the SSR marker MET16-ssr. The palms with *Vir* fruits matched the homozygous (top segregating band present) or heterozygous (both the segregating bands present) profile of the probe. The palms with *Nig* fruits showed a profile consistent with only the bottom-segregating band present (homozygous for the alternative allele).

The linkage of the SSR marker MET16-ssr to the *Vir* trait was tested on independent crosses with genetic backgrounds different from that of the mapping population used to generate the marker. The results are presented in *Figure 4* and the marker could differentiate the *Nig* and *Vir* fruits in all of the crosses tested with about 95% accuracy.

NOVELTY OF THE TECHNOLOGY

The use of the marker MET16-ssr for differentiating fruit colour represents the first successful application of SSR markers in oil palm breeding. This technique can be used to select palms for the desired fruit colour as early as in the nursery, long before they fruit.

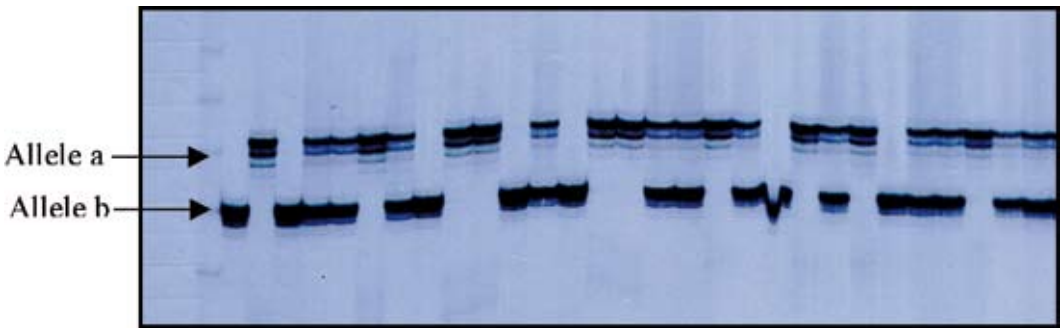

Figure 3. Screening of the SSR marker MET16-ssr linked to the fruit colour locus. Two alleles are segregating in the samples. The presence of alleles aa (homozygous) and ab (heterozygous) indicates the Vir *trait, while the appearance of alleles bb indicates* Nig.

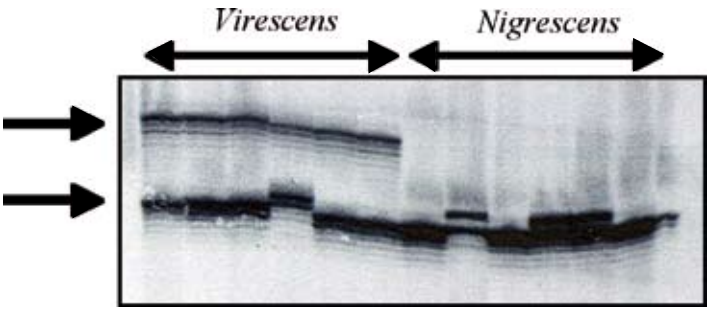

Figure 4. Screening of the marker MET16-ssr linked to the fruit colour trait on an independent population (DxT38). Arrows indicate the bands differentiating Vir *and* Nig *fruits.*

APPLICATIONS OF THE MARKER

- The marker can be tested using leaf samples, indicating that it could be used as a selection tool in the nursery prior to field planting;
- The marker is co-dominant, thus, it is able to distinguish the heterozygous and homozygous forms of the *Vir* trait. Oil palm breeders can incorporate the homozygous forms of the dominant *Vir* trait into their breeding populations in order to avoid any further segregation of the trait; and
- In breeding programmes involving non-abscising genotypes, the marker can expedite the creation of new oil palm varieties which have the potential to decrease the labour required for harvesting and increase OER.

PRICING AND ECONOMIC FEASIBILITY

The analytical service for differentiating oil palm fruit colour using the SSR marker is offered either as a service or technology transfer.

Fruit Colour Analytical Service
Break-even analysis for this service by MPOB at a capacity of 300 samples per year indicates a minimum price of RM 600 for a set of five samples, with each sample tested with the microsatellite primer pair.

Technology Transfer
The technology can be licensed to interested parties under terms negotiable with MPOB.

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