MARKERS TO PREDICT SKIN COLOUR OF OIL PALM FRUIT

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The collection of loose fruits (an indicator of bunch ripeness) is an important practice towards maximizing yield in oil palm plantations. This activity can take about 28% of the total harvesting time (Hitam *et al.*, 1999). 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 external colour of oil palm fruits before and after ripening can be exploited to reduce loss of yield through inefficient collection of loose fruits. Oil palms can be distinguished based on the colour of their fruit coats. The two most common types are *nigrescens* (*Nig*) and *virescens* (*Vir*) palms (*Figure 1*). It is this difference in the colour of the coat that has the potential to be exploited for improved yields. *Nig* fruits are deep violet to black at the apex and pale greenish yellow at the base when unripe, with minimal change in colour on ripening. Harvesters usually rely on fallen detached fruits on the ground to determine the ripeness of the bunch for harvesting. *Vir* fruits, on the other hand, are green when unripe, and change to light reddish orange on maturity. Since the *Vir* fruits undergo a distinct colour change on ripening, it is easier for harvesters to identify the ripe bunches. The *Vir* trait is monofactorial and dominant. As such, identification of its allele and introgressing into non-abscising genotypes will allow easier identification of the ripe bunches with reduced crop loss from fallen fruits (Jack *et al.*, 1998). This can be expedited via marker-assisted selection or MAS.

MARKER-ASSISTED SELECTION (MAS)

MAS is defined as selection of desirable genotypes in conventional breeding aided by DNA-based diagnostics. The main advantage of MAS is that the plants can be differentiated early by their DNA, and hence, selected early, even before the characteristic in question is manifested visually. This greatly reduces the time required for bringing new varieties to market (Mazur and Tingey, 1995). The basic requirement for a MAS programme is the availability of genetic linkage maps on which markers associated with the selected traits of interest (in this case the fruit colour gene) can be located.
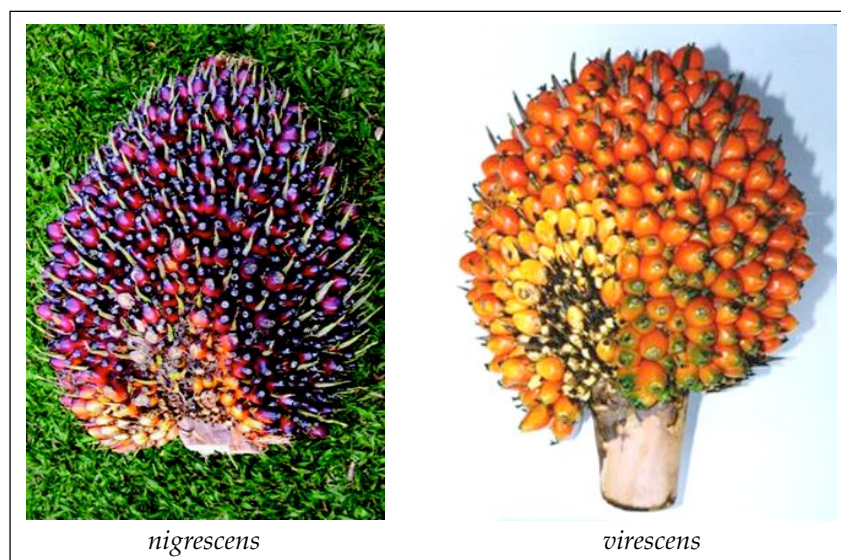

nigrescens   *virescens*

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

IDENTIFICATION OF MARKERS CLOSELY LINKED TO THE FRUIT COLOUR TRAIT

The fruit colour gene in oil palm was located on a genetic map constructed using a population segregating for the trait (*Figure 2*). Two interesting markers, MET16 and KT3, linked to the fruit colour gene were identified. Both MET16 and KT3 are RFLP probes which show co-dominant profiles. *Figures 3a* and *b* display a subset of the mapping population analysed with probes MET16 and KT3, respectively. Majority of palms with *virescens* fruits (93%) matched the homozygous (top segregating band present) or heterozygous (both the segregating bands present) profile of the probes. At the same time, a large proportion of the palms with *nigrescens* fruits showed a profile consistent with only the bottom segregating band present (homozygous for the alternative allele). *Table 1* further illustrates that both probes show the expected 1:2:1 ratio for the fruit colour characters; *virescens* (homozygous for one allele): *virescens* (heterozygous form): *nigrescens* (homozygous for the second allele). Both probes thus show promise as markers for differentiating the homozygous and heterozygous forms of the *virescens* character.

The linkage of probes MET16 and KT3 to the *virescens* trait was tested in other independent crosses with genetic backgrounds different from that of the mapping population used to generate these markers. The results are presented in *Figure 4* and *Table 2*. Both probes could distinguish the *nigrescens* and *virescens* fruits in all of the crosses tested with about 95% accuracy.

NOVELTY OF THE TECHNOLOGY

The use of the MET16 and KT3 probes for distinguishing fruit colour represents the first successful application of MAS in oil palm. This technique can be used to select palms for the desired fruit colour as early as in the nursery, even before they fruit.
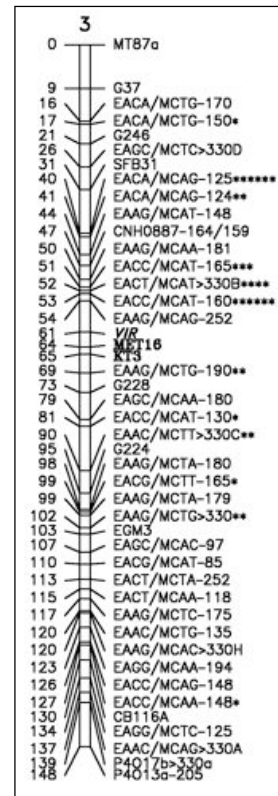

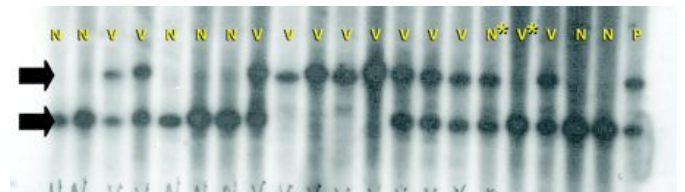*Figure 2. Mapping of the fruit colour gene* (Vir).

Figure 3a. Segregation of the marker MET16 compared with that of the fruit colour trait in the mapping population. N is nigrescens *and V is* virescens *fruits.*
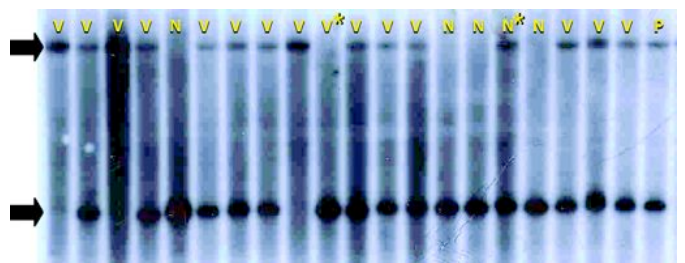** Indicates fruit colour not matching the banding profile.*

Figure 3b. Segregation of the marker KT3 compared with that of the fruit colour trait in the mapping population. N is nigrescens *and V is* virescens *fruits.*
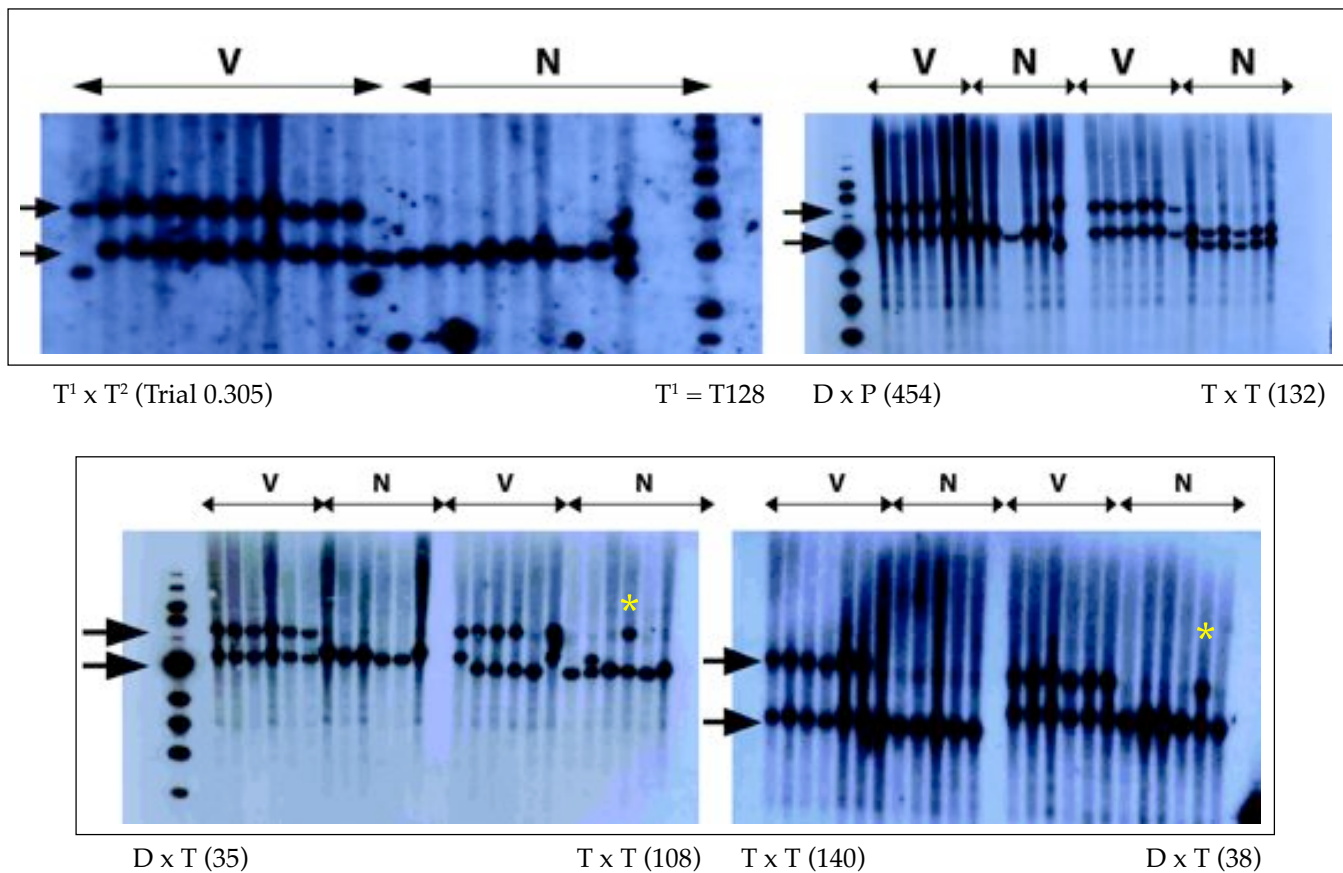** Indicates fruit colour not matching the banding profile.*

Figure 4. Screening of the marker MET16 linked to the fruit colour trait on seven independent populations.
V and N segregation refer to virescens *and* nigrescens *fruits, respectively.*
Arrows indicate the bands distinguishing virescens *and* nigrescens *fruits.*
*Denotes palms showing a mismatched with the expected profile due to recombination
between the marker and fruit colour locus.

TABLE 1. CORRELATION OF *Virescens* AND *Nigrescens* PALMS WITH EXPECTED BANDING PROFILE FOR PROBES MET16 AND KT3

Probe: MET16

No. of palms with field data	V	V	N	χ^2	No. of palms with fruit colour not matching banding patterns	
	- - (1)	- - - (2)	- - (3)		*Vir*	*Nig*
125	28	62	21	2.40	1	4

Note: * No. of palms for which the RFLP profile was not clear / missing data: 9.

Probe: KT3

No. of palms with field data	V	V	N	χ^2	No. of palms with fruit colour not matching banding patterns	
	- - (1)	- - - (2)	- - (3)		*Vir*	*Nig*
125	28	60	24	0.86	3	5

Note: * No. of palms for which the RFLP profile was not clear / missing data: 5.

TABLE 2. LINKAGE OF RFLP MARKERS, MET16 AND KT3, TO THE FRUIT COLOUR LOCUS IN SEVEN FAMILIES

No.	Family	Number of progeny palms	Number of palms matching expected profile		Number of recombinants	
			MET16	KT3	MET16	KT3
1	T¹ x T²	21	21	21	-	-
	T¹ = T128					
2	D x P (45)	12	11	11	1	1
3	T x T (132)	12	12	12	-	-
4	D x T (35)	12	12	12	-	-
5	T x T (108)	12	10	11	2	1
6	T x T (140)	12	12	12	-	-
7	D x T (38)	12	11	11	1	1
Total		93	89	90	4	3

APPLICATIONS OF MARKERS

- The markers can be tested using leaf samples indicating that they could be used as selection tools in the nursery, prior to field planting.

- The markers are co-dominant, that is, they are able to distinguish the heterozygous and homozygous forms of the *virescens* trait. This is extremely useful for oil palm breeders interested in incorporating the homozygous forms of the dominant *virescens* trait into their breeding populations in order to avoid any further segregation of the trait.

- When used in breeding programmes involving non-abscising genotypes, the markers will 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 distinguishing oil palm fruit colour using the MET16 and KT3 probes can be offered either as a service or a technology transfer.

Fruit Colour Analytical Service

The service for fruit colour prediction in MPOB's laboratory has been priced using break-even analysis. At a capacity of up to 500 samples per year, the analysis showed that the price which should be charged must be at least RM 6000 for a set of 25 samples, each sample being tested with both of the probes using two restriction enzymes.

Technology Transfer

The technology can also be licensed. A capital cost of about RM 1 million will be required to set up a properly functioning laboratory. For the establishment of a laboratory with capability of analysing 1000 samples per year, discounted cash flow (DCF) analysis showed that the project is viable if the price charged is above RM 12 500 per set of 25 samples.

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