

BREEDING *Virescens* OIL PALM

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ABSTRACT

Recent discoveries of five independent but closely related nucleotide mutations that result in the *virescens* fruit type in oil palm, and their diagnostic markers, have renewed interest to breed for the trait. In *virescens* palms, the immature fruits are green, ripening to a bright orange, whereas in the common *nigrescens* palms, the immature fruits are a deep purple, almost black, and ripen to red with purple tinges. Ripe *virescens* bunches are more easily spotted, especially at distance and through the lower fronds and epiphytes on the trunk, thus, having fewer missed in harvesting. Correspondingly, unripe and under-ripe *virescens* fruit bunches would be apparent, compared to their *nigrescens* counterparts, during fruit milling. While diagnostic markers will improve the breeding efficiency and save on time and costs, starting with the right *virescens* palms will ensure that the trait is not gained at the expense of yield.

Keywords: breeding, milling, *nigrescens*, oil palm, ripening, *virescens*.

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INTRODUCTION

There are two fruit colour types in the African oil palm (*Elaeis guineensis*) – the usual *nigrescens* and the much rarer *virescens*. In *nigrescens* (Latin for black), the unripe fruits are a deep-violet, seeming black, ripening to red with some residual violet in the apical and cheek regions. In *virescens* (Latin for green), the fully green young fruits ripen to a bright orange with just a little green on the apex (Figure 1). *Virescens* just lack the epicarp anthocyanins and other flavonoids (hence, the absence of dark colours) and have no other known differences with *nigrescens*, for example, higher/lower yield or oil quality. While the ripening skin colour change in *nigrescens* results from the degradation of anthocyanins and other flavanoids (Hazir *et al.*, 2012) and chlorophyll (Ikemefuna and Adamson, 1984) (the underlying red coming through these diminishing masking colours), it is largely the degradation of chlorophyll in *virescens* and the underlying orange coming through, especially with

the accumulation of carotenoids in the mesocarp (Hortensteiner and Krautler, 2011). As the colour change is gradual, bunches of intermediate colours may be found on the same palm. Though less obvious, intermediate colours may also be discerned in the fruits of the same bunch, reflecting their differences in development from a common pollination time, for example, green parthenocarpic fruits in an otherwise bright orange ripe *virescens* bunch (Figure 2).

The evolutionary implication(s) for the colour difference is unknown as both types seem equally attractive to dispersal agents, such as birds and small mammals, nor have any advantage/disadvantage from biotic or abiotic pressures. Logically, *virescens* should occur more, perhaps even exceed *nigrescens* in some locations. This is because it is genetically dominant over *nigrescens* and, while *virescens* mutations are rare (see next section), over an evolutionary period of 6-51 Mya (Ergo, 1997; Singh *et al.*, 2013) and with clear human preference for it, it would have, arguably, overtaken *nigrescens*, at least in some locations. So, its continued rarity suggests some unknown factor(s) culling it, possibly anthocyanins affording *nigrescens* some protection. But this is entirely speculative as also the suggestion that the mutations may be all very recent (Rao, 1987).

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Figure 1. Ripe *virescens* bunch showing orange fruits with halos of green at the apex, pale yellow where less exposed to the sun.



Figure 2. Ripening *virescens* bunch showing fruits of intermediate colours and green parthenocarpic fruits.

Virescens TRAIT

Rao (1998) observed fruit and bunch ripening in *virescens* palms. The bunches are green until about two to three weeks before the onset of fruit abscission. Then the colour rapidly changes to brass/bronze-green, then to brass/bronze-orange, to increasingly orange and, finally to a bright reddish-orange. In a palm, bunches of all the above colours may be seen, reflecting their different ages. To a smaller extent, the colour gradation also occurs in the individual fruits of a bunch reflecting the small differences in development despite their near-simultaneous pollination.

Oil accumulation occurs with the colour change but the rapid increase in the final weeks is when the fruit is already largely orange. The accumulation is complete just before or at first fruit abscission. Hence, while bunches that are mostly orange may already have much oil, the maximum is only reached at incipient fruit abscission. In other words, for maximal oil the indicator for when to harvest ripe bunches can still be the normal *nigrescens* standard of counting loose fruits. This detracts somewhat from the appeal of *virescens*, but the trait is nevertheless still useful as the harvester is more likely to miss darker bunches in the darker recesses of the palms at height. It will be even more important when the loose fruit standard cannot be followed, for example, when harvesting labour is short or during the monsoon floods. Also, *virescens* will avoid the harvesting conundrum from 'physiological ripening' where young bunches with little oil drop fruits from softened, watery ends, the causes of which are still unknown.

In the *virescens* fruit, the colour change is acropetal (from base to top). In the bunch, the colour change is also acropetal, with fruits in the bottom spikelets leading the way, following the also acropetal flower anthesis. However, in many bunches, this acropetal pattern is obviated by other influences and no pattern is obvious. As abscission of the ripe fruits commences when most of them are still brass/bronze (not yet orange), the development of more-uniform ripening palms is key. This trait is more readily scored in *virescens* than *nigrescens*, and can be done in the bunch analysis laboratory.

Like in *nigrescens*, unexposed (to light) fruits or parts of them in *virescens* are a lighter hue - pale cream when young, with an orange tinge on ripening. Parthenocarpic fruits are less bright and their change in colour slower. This is also influenced by the extent of parthenocarpy in the spikelet/bunch - the more parthenocarpy, the slower the development and colour change. This slower colour change is, in fact, a good visual indicator of the pollination. If the harvested bunch is largely orange but with many interspersed green fruits, then the pollination is poor. Where there is an extensive pollination problem, in young or some clonal fields, for example, the overall bunch colour may be arbiter of whether to send it for milling or discard.

Rao (1998) also observed that harvested under-ripe bunches continue their colour change, and this may be temptation to 'age' them to 'full' ripeness, but the oil will not increase.

Genetics

Early work in Congo and Nigeria suggested that a single dominant gene causes *virescens*. In Congo, a *virescens* palm gave 75% *virescens* in its selfs while out-crossing to *nigrescens* gave 50% each of *virescens*

and *nigrescens* (Beirnaert and Vanderweyen, 1941). Hartley (1988) reported that in Nigeria an open-pollinated *virescens* bunch gave 46% *virescens* (and 54% *nigrescens*) and nine *virescens* x *nigrescens* crosses gave 54% *virescens* and 46% *nigrescens*, that is, about 50:50. He also made the interesting observation that in *virescens* the absence of anthocyanins (of which there are several types) is not absolute, with traces of one/some which may be distinct from those in *nigrescens*.

Singh *et al.* (2014) discovered that mutations of the (Vir) gene render it dysfunctional for anthocyanin biosynthesis, that is, they cause *virescens*. Three single nucleotide mutations, a deletion and a nucleotide rearrangement, collectively accounted for 99% of the *virescens* phenotypes, but the 1% discordance suggests that there may be yet other mutations or mechanisms. The small discordance is, however, academic for practical *virescens* breeding which can now be much more efficient. Furthermore, as mutations of the Vir and Shell gene are independent, *virescens* can occur in *duras*, *teneras* and *pisiferas*. Hence, good *virescens* DxP can be produced from an elite homozygous Vir *pisifera* through conventional breeding aided by the genotyping tools.

The mutations occur very rarely, as evidenced by the low frequency of *virescens* found – 0%-3% in Ivory Coast (Meunier, 1969), 0.5% and 0.7% in Nigeria and Angola, respectively (Hartley, 1988), and 6% and 0.7% in Cameroons and Zaire (now known as the Democratic Republic of Congo), respectively (Rajanaidu, 1986). Similarly, amongst the millions of cultivated oil palm in Southeast Asia, only an occasional *virescens* is seen, presumably not from the sources of planting materials. The low frequency also implies that most of the mutations are in the heterozygous state.

The frequencies are higher with human interference, for example, the 6% in Cameroons above is an average from about 2000 wild palms in 11 sites, spiked by two more cared - for sites with 18% and 36%. In many parts of Africa, the 'red palm fruit', or Akwu Ojukwu, is revered, its oil and kernel believed to cure ailments and ward off evil (Eziokwu Chineke Gadi, 2017), valued as an anti-poison and miracle oil in Igboland traditional medicine (Ogbuanu *et al.*, 2015) and a general treatment for illness in rural Benin (Akpo *et al.*, 2012), *etc.* Perhaps, as in humans, redheads are preferable to blackheads, even without any lore of the occult, simply because they are more exotic.

Usefulness of *Virescens* Trait

The most cited use for the trait is as a cue for bunch ripeness, to tell when to harvest it. The colour change with ripening is more distinct in *virescens* than in *nigrescens*, making ripe bunches easier to spot, particularly in tall palms, through

the maze of lower fronds and axillary epiphytes on the trunk. Similarly, ripeness needs to be gauged in the fresh fruit bunches (FFB) landed at the palm oil mill to decide on which to process/discard. With ripeness being the most important determinant of oil extraction rates, the economic implication of accurately determining it is substantial. The traditional method is to sight a specified number of abscised, or 'loose', fruits fallen from the ripening bunch. But, interest in mechanised harvesting with increasing worker shortage, is veering it to more automated assessment, particularly vision- or image-based.

Real-time automated assessments, from which mechanical segregation can be effected, may be even more feasible in the palm oil mill. Some exploratory work on automated assessments can be found in Abdullah *et al.* (2001); Alfatni *et al.* (2008; 2014); Bensaeed *et al.* (2014); Cherie *et al.* (2015); Hazir *et al.* (2012); Junkwon *et al.* (2009); Roseleena *et al.* (2011); Saeed *et al.* (2012); Tan *et al.* (2010) and Utom *et al.* (2018).

The discovery of non-abscinding/non-oil palm (Donough *et al.*, 1995) offered hope for needing less of the laborious and costly manual loose fruit picking off the ground. But the counting of dropped/loose fruits from ripening bunches is now the criterion for harvesting them, so how to tell bunch ripeness in such palms? By incorporating the *virescens* gene into them! Crosses between non-shedding *teneras*, and a selected *virescens tenera* and *dura*, both ex-Lobe, Cameroon, were planted in 1999 in Pamol (Rao *et al.*, 2001). Meanwhile, the pioneering work on oil palm fruit abscission by Henderson and Osborne (1990; 1994) has continued with further insights into related anatomical and biochemical changes (Henderson *et al.*, 2001; Roongsattham *et al.*, 2016); Tranbarger, 2012 and their genetical control (Fooyontphanich *et al.*, 2016; Roongsattham *et al.*, 2012; Tranbarger *et al.*, 2019).

Ogbuanu *et al.* (2015) reported that while both *nigrescens* and *virescens* oils have similar physical properties, the latter, with an iodine value of 83.8 is closer to olive oil and likewise too its density. The peroxide value of *virescens* oil is also markedly higher than in *nigrescens* oil, as would be expected if it is less saturated. The more than double phospholipids and presence of cystine (an amino acid) are suggested to confer the medicinal and anti-poison properties of *virescens* oil. However, there has been little corroboration of Ogbuanu *et al.* (2015) results, which may, therefore, be spurious. Other studies have shown the iodine values of both oils to be similar. For example, in their attempt to produce high iodine value material, the Malaysian Palm Oil Board (MPOB) screened ~2400 palms from the Malaysian Agricultural Research and Development Institute (MARDI)-Nigerian Institute for Oil palm Research (NIFOR) prospection of 1973, including

several *virescens* palms. The maximum iodine value from a *nigrescens* palm was only 69.75 (Arasu *et al.*, 1988), while *virescens* Palm T128, distributed to the industry for its high iodine value, had 63.4 (Kushairi *et al.*, 1999), not very different.

The breakdown of chlorophyll is good for palm oil as it adversely affects the oil oxidative stability, bleachability and hydrogenation. Ikemefuna and Adamson (1984), in Table 1 and Tan *et al.* (1997) showed that chlorophyll in palm oil decreases with ripening although never completely. As chlorophyll is removed in refining crude palm oil (CPO) for consumption, less of it in the initial oil is better. The question begged is how similar the chlorophyll breakdown processes are in both *virescens* and *nigrescens* oils, and their levels ex-palm, if *virescens* is to become commercial planting material.

***Virescens* FOUNDER PALMS AND POPULATIONS - HISTORIC**

Due to their rarity and no perceived commercial advantage, at least until recently, there has been little interest in *virescens* breeding. However, the progenies of individual *virescens* palms, selected for their other traits, have been exchanged, the fruit colour but incidental. The below listing of the main *virescens* palms exchanged and the diaspora of their descendants is a history of the passing interest in the trait. There was some love for them but not quite the ardour of Romeo and Juliet (Shakespeare, 1597).

NIFOR *Virescens* via Department of Agriculture of Malaya (DOAM)

This is the most distributed *virescens* internationally and over the longest period. The palms, together with the early NIFOR [formerly known as the West African Institute for Oil Palm Research (WAIFOR)] breeding materials, were from 4.45 ha (11 ac) plot of about 800 palms in Calabar, Eastern Nigeria, planted in 1912-1916 from a small number of open pollinated bunches of various types and forms, including mantled and *virescens*. Each type and form was represented by seeds from a single parent. Hence, the occurrence of both *tenera* and *dura virescens* in a particular progeny suggests that the parent was a *tenera virescens*. Yield and bunch data were collected from 1922-1928, and among the nine *duras* selected for performance, two were *virescens* – CA551.341 and CA551.375. Broekmans (1957) provided data on all nine, reproduced in Table 2 with the two *virescens* highlighted. Hartley (1988) mentioned that, besides the two *virescens duras*, some *tenera virescens* were also selected (as seed parents). Specific information on the *virescens teneras* is, unfortunately, not available, but the mean performance of all the 10

selected *teneras* (from 43 *teneras* - 36 *nigrescens* and seven *virescens*) gives an idea of the quality of the NIFOR *virescens teneras* (last row in Table 2). In other words, as the selection was based on performance *per se*, the *virescens teneras* were unlikely to be very different from the overall mean.

The NIFOR *virescens* arrived in Malaya in the early 20th century, at a time of increasing interest in oil palm. The first *virescens* in Malaya was probably from the 1926-1927 introduction by DOAM - 28 palms from "... no less than 40 different lots of seeds from the various palm oil producing countries in West Africa." - established in a ha (1 ac) plot at the Central (later Federal) Experiment Station, Serdang (Bunting *et al.*, 1927; 1934). They recorded that among the more distinct types was *E. guineensis* var. *rapanda* Chev. with fruits that were a "... vivid cypress-green in the early stages of development changing when ripe to a deep orange." This plot, Field 19, included the fertile *pisiferas* - 29/36 and 36/21 - used by DOAM to produce its early DxP planting materials. Given that Malaya and the then British West African countries were fellow British colonies, most oil palm materials came to Malaya through this channel. Thus, early Malayan *virescens* were highly likely to be of NIFOR origin.

The analysis of 100 ripe *rapanda* fruits, presumably from the same bunch, in comparison to the then average Malayan fruit, which was *Deli dura*, are given in Table 3 (Bunting *et al.*, 1934). The constituents, in percentage, suggest that the *rapanda*, or *virescens* fruits were *tenera*, not atypical of the 'wild' *teneras* in Africa. They had a fresh mesocarp content of ~68%. The % shell and % kernel were high compared to modern *teneras*, but lower than in *dura* then and now.

From this African/NIFOR introduction, a selected *virescens tenera* was crossed to fertile *pisifera* 29.36 from the previously-mentioned Field 19, and twice to a selected *virescens tenera* from the nearby Highlands Estate (HE). These three *virescens* families, together with another seven miscellaneous TxT families, were planted in Trial 0.126 at the Federal Experiment Station (FES) Serdang in 1969. We have no record of the HE *virescens* palm but, given that most of the early materials at HE were from FES Serdang, it may have been a descendant of the Field 19 *virescens*. A cross of the HE *virescens* with Serdang fertile *pisifera* 29.36 was provided to Federal Land Development Authority (Felda), later transferred to Felda Global Ventures (FGV), when they started their breeding programme, the cross coded as progeny 'RM'. The only other early *virescens* in the country then, from records we have sighted, was an Ulu Remis *virescens* which is described in the next section. Gray and Bevan (1966) mentioned that *virescens* was quite rare in Malaya and that there was no commercial interest in it.

TABLE 1. REDUCTION IN CHLOROPHYLL CONTENT (mg kg⁻¹) IN OIL OF RIPENING *Nigrescens* FRUIT*

Pigment	Fruit type	<i>Tenera</i>			<i>Dura</i>		
	Ripeness	Green	Mature	Ripe	Green	Mature	Ripe
	Age (months)	1-2	3-4	5-6	1-2	3-4	5-6
Chlorophyll a		28.9	20.7	4.3	26.5	22.7	2.4
Chlorophyll b		18.6	15.3	7.3	19	11.8	4.6

Source: * Ikemefuna and Adamson (1984).

TABLE 2. QUALITY OF NIFOR *Duras* SELECTED FOR PERFORMANCE, INCLUDING TWO *Virescens*, AND OF *Teneras* INCLUDING SOME *Virescens*

Parent palm	FFB (kg yr ⁻¹)	FB (%)	MF (%)	LOSS (%)	SF (%)	KF (%)	FWT (%)
<i>Dura</i>							
551.141	64.9	78.3	46.2	3.4	32.9	17.5	11.3
551.222	75.7	70.8	52.4	3.4	32.1	11.9	8.2
551.224	77.1	67.9	43.9	3.1	39.1	13.9	8.6
551.233	90.7	58.7	48.2	2.9	34.8	14.1	4.5
551.256	114.3	64.7	52.5	2.7	30.4	14.4	9.1
551.341	82.6	67.8	49.3	4.4	32.7	13.6	12.2
551.375	139.3	63.4	44.2	4.7	38.7	12.4	6.4
551.703	112.5	72.8	43.8	3.4	40.0	12.8	10.9
551.261	61.2	68.6	62.6	2.1	22.2	13.0	11.3
<i>Tenera</i> (including <i>virescens</i>)							
Average	67.0	56.0	64.0	-	18.0	10.0	-

Note: FFB - fresh fruit bunch yield in kg palm⁻¹ yr⁻¹; FB - % fruit in bunch; MF - % mesocarp in fruit; LOSS - % material loss during analysis; SF - % shell in fruit; KF - % kernel in fruit; all % on fresh weights; FWT - mean fruit weight.

Source: Broekmans (1957).

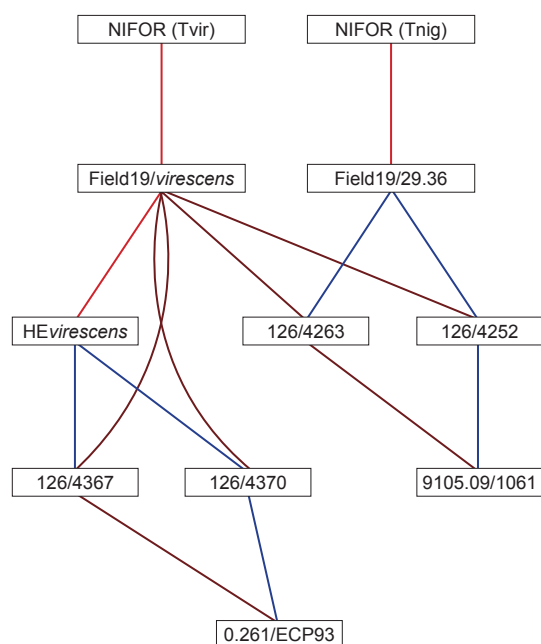
TABLE 3. COMPARISON OF AVERAGE MALAYAN OIL PALM FRUIT (Deli *Dura*) AND RAPANDA TYPE (*Virescens*) *Tenera** IN 1930s

Constituent	Average Malayan fruit	Rapanda type
Palm oil	29.0	32.0
Palm kernel	6.0	9.0
Shell	30.0	23.0
Moisture and residue	35.0	36.0

Note: *Bunting *et al.* (1934). All figures are % of fresh fruit weight.

The third generation (with the original *virescens* in Africa as the first) *virescens* comprised selected palms from Trial 0.126, sib-mated and planted in Trial 0.261 at Bukit Lawang, Johor, Malaysia in 1990. This was undertaken by MPOB which, since its inception in 1979 as the Palm Oil Research Institute of Malaysia (PORIM), managed the oil palm trials at FES Serdang while founding new oil palm research stations. Some of

the MPOB crosses were also provided to Eastern Plantation Agency (EPA), which had then just started oil palm breeding (Rao and Musa, 1995), and planted in Trial 9105.09 at Ladang Tereh Selatan (LTS) estate. The MPOB crosses may still be extant but the EPA trials have probably been replanted. Figure 3 shows the descent, from 1920s-1990s, of the first *virescens* that entered Malaya/Malaysia.



Note: NIFOR - Nigerian Institute for Oil Palm Research.
HE - Highlands Estate.

Figure 3. Lineage of ex-NIFOR Serdang virescens, 1920-1990.

Via IRHO/CIRAD

As mentioned above, Felda/FGV received NIFOR *virescens* through DOAM in the late 1960s. Some five years later, more NIFOR *virescens* were received from the Institut de Recherches pour les Huiles et Oleagineux (IRHO) [now Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD)] from their stations in Pobe, Benin and La Me in Ivory Coast. There were eight crosses, two between *virescens* palms and the rest *nigrescens* x *virescens*.

United Plantations Berhad (UPB) planted two *virescens* progenies in 1977. The first (TT69) was an early Deli *dura* (ex-Marihat Baris) crossed to a Yocoboue selection from IRHO with the *virescens* probably the latter. The other progeny (TT80) was derived from an ex-NIFOR *virescens tenera* with IRHO code WA10. *Teneras* and *pisiferas* from TT69 and TT80 are prominent in UPB's subsequent crosses but *virescens* individuals rare. A selected palm (*virescens*) from TT69 was crossed to a selected palm from TT1 (L239TxL432T) and the progeny (TT132) planted in 1992.

Ulu Remis *Virescens*

The second batch of *virescens* distributed was among the first oil palms planted at Ulu Remis Estate, Johor. They were open-pollinated seeds from Sumatra, believed to be from Marihat Baris Estate (Rosenquist, 1999). While the Marihat breeding

programme then focussed on breeding only the Deli *dura* type (all *nigrescens*), imported African seeds were also planted around 1920 (Janssen, 1959). Hence, while it is possible that the Ulu Remis seeds came from yield-recorded Deli palms, the fact that they were open-pollinated suggests the *virescens* to be *Nigrescens*_{Deli} x *Virescens*_{African}. Nevertheless, the possibility of it being a Deli *dura* mutation from *nigrescens* to *virescens* cannot be dismissed. The *virescens* was among 175 selected, with FFB>200 kg yr⁻¹, as parents for commercial seed production and further breeding. The palms were labelled 'PP' and the *virescens* was PP201.

A self of PP201 was planted in Trial GB4B in 1940 (and as supplies in Trial GB1A). The self was UR258 and, 20 years later, two *virescens* palms (UR258/1 and UR258/2) were selected from this family to create *virescens* UR672 and 673, the first a sib cross and the second an outcross to a *nigrescens* (UR120/2). Besides planting in Ulu Remis (Trial GB19B), UR672 was provided to UPB and Société Financière des Caoutchoucs (SOCFIN) (coded SOC2739). The cross was planted in 1959/1960 at these three locations but no resulting *virescens* palms seemed to have been selected for further use.

Virescens FOUNDER PALMS AND POPULATIONS - RECENT

All the recent *virescens* palms in Malaysia came from germplasm collected in the 1970s and 1980s. *Virescens* was encountered in most of the countries prospected, and collected if ripe bunches were available. From these open pollinated collections, heterozygous for the trait, a few individuals were selected for breeding, although not for their *virescens*. The unselected *virescens* have since been discarded.

Palm 0.151/128T and Discovery of the *Virescens* Gene

The most disseminated and tested recent *virescens* palms are all from the MARDI-NIFOR prospections in 1973. The 1973 prospection in Nigeria was not only the most systematic search but its provenances also the best reconnoitred. The germplasm was planted at the MARDI station in Kluang, Johor, Malaysia between 1975-1976 and handed over to MPOB in 1979. Crosses from three *virescens* palms, from that germplasm, have been distributed to the industry but, again, not for their *virescens*. Of them, 0.151/128T is the most disseminated and tested.

Oil (un)saturation was the rage in the 1980s, for the now debunked coronary health reasons, and for a more liquid palm oil. The existing germplasm was obviously the best place to search. Trial 0.151 at MPOB Kluang, with palms from the 1973 Nigerian prospection, was a good starting point. Of the ~2400 palms screened, 13 had iodine value >61 compared

to ~55 of commercial materials. Of them, only 151/128T was *tenera* (the rest *duras* as the prospection had focussed on *dura*), and it was also high yielding and *virens*. Table 4 shows some of its performance data as well as those of the population from which it came. Table 5 shows the fatty acid composition of its oil, the primary reason for its selection. The palm was an open pollinated offspring from a *dura virens* bunch from Ufuma, Nigeria. Like much of the prospected materials, the fruits were small, a mere 5 g, with 46% mesocarp (field notes from 1973 MARDI-NIFOR prospection in Nigeria).

Selves of the palm and its pollen were provided to several companies interested in breeding for a less saturated oil, besides being planted in MPOB itself. It was this combined large population of 0.151/128T offspring, segregating for *nigrescens* and *virens*, that helped in the discovery of the *virens* gene.

UPB was upfront in the breeding for less saturated oil, hence, their interest in 0.151/128T, not for its *virens*. The other high iodine value selections acquired by UPB from MPOB were all *duras* and *nigrescens*. Besides the selfing of

0.151/128T, it was also crossed to 10 UPB high iodine value *teneras*, three *pisiferas* from high iodine value families, an *oleifera* and *oleifera-guineensis* hybrid. Sharma (1999; 2003) presented their performance (including iodine value), followed by an update by Musa and Gurmit (2008) for the *E. guineensis* crosses (Table 6). The selves retained the high iodine value of their parent and the small fruits and bunches, but the oil yields were low. Outcrossing with UPB high iodine value selections improved the fruit and bunch size but with still low oil yields and some diminution in the iodine value. The values for the crosses with the *Oleifera-guineensis* hybrid and an *Oleifera-guineensis* × *guineensis* backcross were typical for such crosses with no additional value conferred by the high iodine value 0.151/128T. Nevertheless, a few individuals with oil yield comparable to that of commercial DxP were identified for cloning.

Like most, if not all *virens*, 0.151/128T was heterozygous for the trait and its selves and outcrosses segregated into *nigrescens* and *virens*. Three palms from the selves and one from an outcross were selected for further crossing and their descendants shown in

TABLE 4. PERFORMANCE OF PALM 0.151/128T AND ITS BACKGROUND POPULATION

Pop or palm	FFB	BN	BW	FW	FB	MF	OM	OB	KB
Pop 14 (T)	174.4	16.6	11.4	6.8	69.4	80.8	50.9	26.5	5.5
Pop 14 (D)	166.3	15.4	12.3	9.6	64.6	49.6	48.4	15.6	7.6
Palm 0.151/128T	217.2	22.3	10.3	7.2	67.5	80.8	50.0	27.2	4.9

Note: FFB - fresh fruit bunch yield in kg palm⁻¹ yr⁻¹; BN and BW - average number of bunches produced / year over the period of recording and their average weight (kg); FW - mean fruit weight (g); FB - % fruit in bunch; MF - % mesocarp in fruit; OM - % oil in mesocarp; OB and KB - oil and kernel content (%) of fruit bunches; all % on fresh weights.

TABLE 5. FATTY ACID COMPOSITION (in %) OF PALM OIL FROM *Virens* PALMS 0.151/128T AND 0.151/618D

Palm	C14:0	C16:0	C18:0	C18:1	C18:2	IV
0.151/128T	0.6	35.3	5.3	42.1	15.8	63.4
0.151/618D	0.6	33.7	6.7	44.2	13.5	61.2

Note: IV - iodine value.

TABLE 6. PERFORMANCE OF MPOB *Virens* 0.151/128T CROSSES AT UNITED PLANTATIONS BERHAD*

Cross	FFB	BN	BW	FW	MF	SF	OB	KB	HI
0.151/128T self	196.0	28.8	5.4	6.7	78.5	13.7	21.5	4.5	0.56
TT29/64x0.151/128T	240.0	26.6	9.0	10.5	82.9	9.0	25.9	6.4	0.49
TP6/328x0.151/128T	241.3	27.9	8.6	6.7	80.3	11.1	23.5	6.0	0.53
TT10/843x0.151/128T	237.5	27.0	8.8	7.2	76.8	12.1	22.9	4.9	0.46
TT10/867x0.151/128T	209.0	30.5	6.9	7.7	76.3	13.8	21.5	4.9	0.45
Standard DP cross	246.5	21.6	14.8	12.9	82.4	8.8	26.8	5.4	0.61

Note: FFB - fresh fruit bunch yield in kg palm⁻¹ yr⁻¹; BN and BW - average number of bunches produced / year over the period of recording and their average weight (kg); FW - mean fruit weight (g); MF - % mesocarp in fruit; SF - % shell in fruit; OB and KB - oil and kernel content (%) of fruit bunches; all % on fresh weights; HI - height increment in cm yr⁻¹.

Source: *Musa and Gurmit (2008).

Figure 4. We are not aware if any of the selections were *virescens* and, hence, whether the *virescens* trait was continued. Note that if the WA10 origin (a NIFOR *virescens*) provided to UPB by IRHO, and its descendant, TT80/45T, as well as TT113/320T from the self of 151/128T, are both *virescens*, then the cross (TT209) would have had both homozygous and heterozygous *virescens* palms. Also, the *virescens* trait in the case would have come from both ancestors, and if the ancestral *virescens* were from different mutations, then the 'homozygosity' would be due to two different Vir alleles and not 2x the same one. While probably of no practical importance, this 'different homozygosity' is godsend for studying the trait genotype-phenotype relationship. A caveat, however, is that Singh *et al.* (2014) found all five *virescens* from the Nigerian germplasm to have the same mutation and the above ancestral *virescens* are from West Africa/Nigeria.

Felda/FGV took the selfs of 0.151/128T and its pollen and used the latter to cross with two Yangambi and one Yangambi-La Me selected *teneras*. The pollen was also used in progeny test combinations with a range of Deli *duras*. An outstanding *tenera* (B36/35T) from a Yangambi cross was extensively progeny tested and used to create new materials with Felda's/FGV's other *tenera*/*pisifera* populations. A sib *dura* (B36/36D) was progeny tested with Yangambi and AVROS *pisiferas*, the crossings illustrated in Figure 4. Besides the above extensive work, Felda/FGV also collected a self of another *virescens* from the MPOB Nigerian collection, high iodine value *virescens dura* 0.151/618D (Table 5), as well as a self of a *virescens dura* (0.221/1362D) from the MPOB Congo collection. These origins, however, were not exploited further.

During this time of active *virescens* breeding several *virescens teneras*, of NIFOR and 0.151/128T origins, were cloned and planted in trials, but not released for commercial planting.

Following their initial, albeit incidental, collection of *virescens* and subsequent extensive work with the Nigerian Prospected Material (NPM) *virescens*, as well as crossings with their advanced Yangambi families, Felda registered two *virescens* 'varieties' in 2009 – Felda *Tenera* Yangambi *Virescence* and Felda *Dura* Yangambi *Virescence* – for protection. Despite Felda/FGV having produced pure *virescens* DxP, there has been no commercial release possibly because their performance is only comparable to that of commercial *nigrescens* DxP. The industry would need more incentive, for example, much higher yields, to depart from their tried and trusted *nigrescens*.

Applied Agricultural Resources (AAR) included 151/128T in their NPM testing with their advanced *dura* and *tenera*/*pisifera* parents (Soh *et al.*, 1999). The palm had good combining ability for high bunch number, high oil/bunch, low height and low frond dry weight. The high iodine value was reflected by high C18:1. However, the work has been discontinued.

The third *virescens* NPM designated for the next generation is 0.150/501T. This is a *tenera* from Population 12, high yielding and very short, and hence with a high bunch index as well as having oil with high vitamin E (Kushairi *et al.*, 1999). Selfs were provided to an industry member besides planting in MPOB. The selfs segregated in the expected ratios for a heterozygous *virescens* and were dwarf, but otherwise poor in yield and bunch characters (Kalaimugilan, 2020).

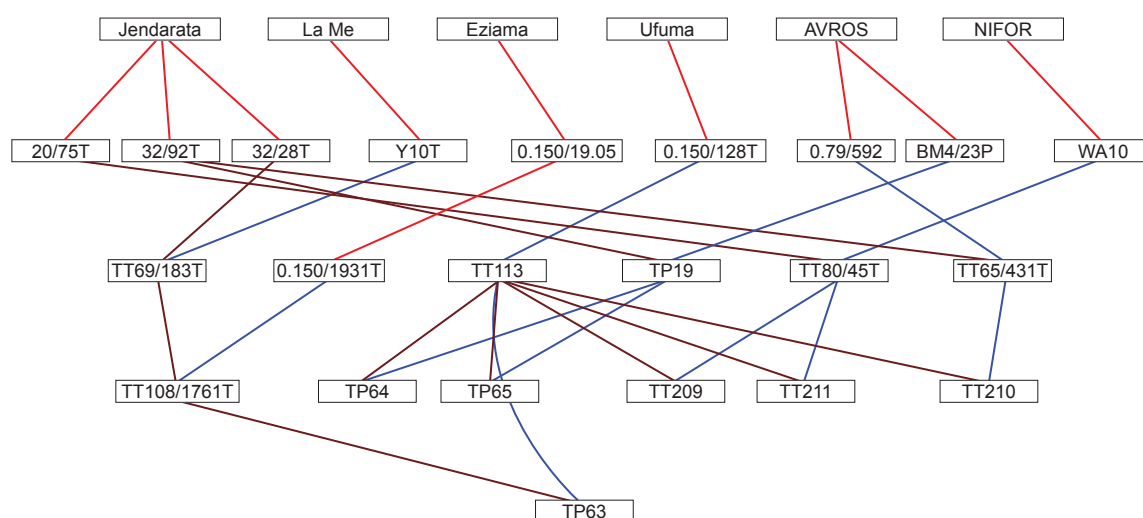


Figure 4. Origins of some high iodine value crosses at the United Plantations Bhd. (UPB) including *virescens* MPOB 0.151/128T and NIFOR/CIRAD WA10.

Angola *Virescens*

The most recent germplasm collection is the 2010 joint prospection by Indonesia, Malaysia and Instituto Nacional do Café de Angola (INCA) in Angola. This was on top of a 1991 limited prospection by MPOB/INCA in the same country. The Angola palms have large fruits and thick mesocarp, in clear contrast to the small poor-quality fruits of other collections, the main stumbling block to their wider use in breeding (Table 7).

Adon *et al.* (1998) showed the successful introgression of the Angola germplasm in the IRHO/CIRAD breeding programme. The work offers hope out of the Deli *dura* genetic bottle-neck, the *raison d'être* for prospection for oil palm diversity in the centres of origin. Furthermore, their (Angola germplasm) tolerance to vascular wilt suggests possible tolerance also to *Ganoderma*, the scourge of oil palm in Southeast Asia.

Following evaluation of its prospected materials, MPOB have disseminated progenies of the best palms to all the major breeding programmes in the country. The average performance of the *dura* and *tenera* palms from the inspections and a selected *virescens* palm provided to the industry is shown in Table 8.

In Indonesia, seedlings from the 2010 prospection have been shared with the sponsors of the expedition - the major oil palm breeding organisations - and the materials, which include

good numbers of *virescens* progenies, are being field trialled.

Interestingly, unlike in the Nigerian germplasm, *virescens* Angola palms could be due to one of three mutant alleles, two point mutations and a deletion (Singh *et al.*, 2014). The difference could be studied for further insight into the trait genotype-phenotype relationship.

CONCLUSION

The common oil palm is the *nigrescens* type which immature fruit is dark violet, ripening to red with violet tinges, following the degradation of anthocyanins, other flavonoids and chlorophyll in the epicarp as carotene accumulates in the mesocarp. The rarer *virescens* lacks anthocyanins and flavonoids, but chlorophyll gives the immature fruit a bright green colour. On ripening, the chlorophyll degrades, and carotenes also accumulate in the mesocarp, making the fruit a bright orange. *Virescens* is due to rare mutations of the *Vir* gene, which make it possible to breed for. The trait is a more obvious cue of fruit ripeness to harvest than *nigrescens*, especially for tall palms, and for grading FFB for milling. It is a vital trait to introgress into non fruit-abscinding oil palm to ascertain when to harvest the ripe bunches which do not shed fruits. Besides employing the newly-discovered genotyping tools for breeding efficiency

TABLE 7. CHARACTERISTICS OF OIL PALM BUNCHES COLLECTED FROM ANGOLA AND OTHER WEST AFRICAN COUNTRIES (all on fresh basis)*

Country	Bunch wt (kg)	Single fruit wt (g)	Mesocarp (%)	Bunch wt (kg)	Single fruit wt (g)	Mesocarp (%)
Ivory Coast	10.9	6.9	41.8	9.8	5.8	61.2
Nigeria	11.8	9.0	47.3	10.9	6.5	70.9
Cameroons	16.8	10.3	39.7	17.3	8.6	62.4
Zaire	17.6	14.2	43.9	17.4	12.6	64.1
Angola	21.4	14.2	48.9	16.0	11.7	70.9

Note: wt - weight.

Source: *Rajanaidu *et al.* (1991).

TABLE 8. MEAN PERFORMANCE OF *Duras* AND *Teneras* IN MPOB'S 1991 ANGOLA PROSPECTIONS AND OF A SELECTED *Virescens* PALM (P0.312/1263T)*

	FFB	BN	BW	FB	FW	MF	SF	ODM	OB	KB	HI
0.312/ <i>Dura</i>	144.4	14.0	10.7	65.0	12.5	49.9	38.7	77.0	15.0	7.4	0.4
0.312/ <i>Tenera</i>	158.6	14.3	11.4	63.1	11.0	74.3	14.2	77.4	21.9	7.3	0.5
0.312/1263T _{vir}	227.9	16.4	14.1	68.2	13.7	72.3	13.9	79.5	23.9	9.4	0.8

Note: FFB - fresh fruit bunch yield in kg palm⁻¹ yr⁻¹; BN and BW - average number of bunches produced/year over the period of recording and their average weight (kg); FB - % fruit in bunch; FW - mean fruit weight (g); MF - % mesocarp in fruit; SF - % shell in fruit; ODM - % oil in dry mesocarp; OB and KB - oil and kernel content (%) of fruit bunches; all % on fresh weights; HI - height increment in cm yr⁻¹.

Source: *Kushairi *et al.* (2004).

gain, starting with the right genetic stocks will shorten the process of achievement. The *virescens* palms from recent germplasm collections in Angola are a promising start. Discovery of the *virescens* gene plus the now widespread trialling of new *virescens* palms, in both Indonesia and Malaysia, may be key to staving off the threats to oil palm – laborious harvesting and fruit collection in the increasingly worker-short industry.

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