Improvement of Palm Oil Through Breeding and Biotechnology[1]

B.S. Jalani*, S.C. Cheah, N. Rajanaidu, and A. Darus

Palm Oil Research Institute of Malaysia, 50720 Kuala Lumpur, Malaysia

ABSTRACT: The oil palm *Elaeis guineensis* is the highest oil-yielding crop and has the potential to become the major supplier of both edible oil and renewable industrial feedstock. The oil yield from wild groves is presently less than 0.5 t/ha/y. However, through breeding and selection, the oil yield of commercial plantations could reach as much as 8 t/ha/y. New planting materials also have the capability of better oil yields with high iodine value (IV), slow height increment, and larger kernels. The oil also contains considerable amounts of carotenoids (500–700 ppm), vitamin E (600–1000 ppm), and sterols (250–620 ppm). The oil yield of another oil palm species, *E. oleifera*, is approximately 0.5 t/ha/y with high contents of carotenoids (700–1500 ppm), vitamin E (700–1500 ppm), and sterols (3500–4000 ppm). The above traits could be improved through breeding and biotechnology. Biotechnological efforts at the Palm Oil Institute of Malaysia are directed toward the production of oil with high IV and high monounsaturated fatty acids for edible purposes and industrial uses. Isolation and manipulation of the genes involved in the biosynthesis of fatty acids are the main focus. The aim is to increase the efficiency of conversion of palmitate ($C_{16:0}$) to oleate ($C_{18:1}$). Levels of palmitate and oleate are controlled by the enzymes acyl-acyl carrier protein (ACP) thioesterase and β-keto acyl ACP synthase II. The chain termination reactions of $C_{16:0}$ and $C_{18:1}$ are independent, thus paving the way for the possibility of reducing palmitate levels by switching off the palmitoyl ACP thioesterase gene.
JAOCS 74, 1451–1455 (1997).

KEY WORDS: Biotechnology, breeding, genetic engineering, oil palm, palm oil.

The center of origin and diversity of oil palm (*Elaeis guineensis* Jacq.) is West Africa, and it has spread to most parts of the tropical and subtropical zones of the world. For example, four palms were planted in Bogor, Indonesia, in 1848. The seeds of these four palms became the base of oil palm expansion in Southeast Asia, including Malaysia, which now accounts for about 52% of the world's palm oil production.

Another species, *E. oleifera*, originates in Central and Latin America, stretching from Guatemala and Honduras in the north to Brazil in the east and Colombia and Ecuador in the west. This species was recently introduced into other parts of the world, including Malaysia, to broaden the genetic base of oil palm and also to exploit its potential. This species normally grows wild, and only a small area is planted for commercial exploitation. However, this species possesses a number of desirable traits, such as slow yearly height increment, high iodine value (IV), and tolerance to some pests and diseases that affect *E. guineensis*.

The oil palm, especially *E. guineensis*, has gone through several cycles of improvement throughout the palm oil-producing countries. Conventional breeding methods have been the main tool with great success. However, with the rapid advancement of molecular applications in plant breeding, strategies have been formulated at the Palm Oil Research Institute of Malaysia (PORIM) to incorporate some of these new techniques into the oil palm improvement programs. This paper will describe the improvement of oil palm through conventional methods and also the application of genetic engineering in breeding programs.

OIL PALM BREEDING

Improvement of E. guineensis. The main emphasis of oil palm improvement is toward higher oil yield, and significant progress has been reported over the last few decades. However, lately, several other traits, such as high IV, high kernel content, slow yearly height increment, resistance to pests and diseases, and minor components (carotenoids, vitamin E, sterol, etc.) have received attention from the breeders. The oil palm yield in the wild groves of Africa is low, averaging 0.2 t/ha/y (1) to 0.8 t/ha/y from improved natural groves (2). Palms in the wild groves do not have the best environment for growth. These groves are either too dense or scattered and are not well fertilized and maintained.

Domestication of oil palm has led to its improvement, especially through breeding. The first step of genetic improvement was based on the discovery of shell thickness inheritance in oil palm (3). When the homozygous dominant, thick-shelled *dura* ($sh^+ sh^+$) was crossed with homozygous recessive shell-less, *pisifera* ($sh^- sh^-$), a 100% heterozygous *tenera* ($sh^+ sh^-$) was produced, which is thin-shelled. Since 1960, all commer-

[1]Paper presented at the 88th AOCS Annual Meeting & Expo, May 11–14, 1997, Seattle, Washington, USA.

*To whom correspondence should be addressed at Palm Oil Research Institute of Malaysia, P.O. Box 10620, 50720 Kuala Lumpur, Malaysia. E-mail jalani@porim.gov.my.

TABLE 1
Average Bunch Composition (%)

Components	*Dura* (Sh^2 Sh^3)	*Tenera* (Sh^2 Sh^3)
Fruit/bunch	60	60
Mesocarp/fruit	60	80
Shell/fruit	30	10
Kernel/fruit	10	10
Oil/wet mesocarp	50	50
Oil/bunch	18	24

cial plantings throughout the world are based on *tenera* planting material. Table 1 shows the improvement of *tenera* oil yield as compared to *dura*. This is a classical case of exploitation of a single gene by which the yield was increased by more than 30%. Before the discovery of *tenera*, the growers largely planted *dura* materials. With the development of *tenera*, oil palm breeding methods have been reviewed, mainly emphasizing combinations of *dura* and *pisifera* to give high-yielding *teneras*. In oil palm breeding, discrete populations of *dura* and *pisifera* for breeding and selection are maintained concurrently to upgrade these populations.

The oil palm FFB (fresh fruit bunch) yield of Deli *dura* populations increased from 17.9 t/ha/y in 1878 to 26.9 t/ha/y in 1969, a rate of 2 kg/palm/y, due to selection progress (Table 2). At the same time, the increase of oil yield was from 3.1 t/ha/y in 1878 to 5.0 t/ha/y in 1969 (4). With one generation of mass selection, selection progress of 23.5 and 19.4% was achieved for FFB and oil yield, respectively (4). Subsequent improvements of 26.3 and 35.1% for FFB and oil yield were achieved with OPRS Deli *dura* in comparison to the first generation of selection. This represents average selections of 8.8 and 11.7 per generation, respectively (4). Using these improved Deli *dura* and also *pisifera* selections, the production of high-yielding *tenera* (*dura* × *pisifera*) as commercial planting materials has been the major emphasis of breeding and selection, especially by the seed producers and research institutions. Table 3 shows the performance of various *tenera* planting materials from 1962 to 1988 (5–7). The oil yield increased from an average of 5.0 to 9.6 t/ha/y, representing an increase of 93.2% and a yearly increase of 3.6% or 0.2 t/ha/y. The performance of the above materials was based on the Deli *dura* that originated from the four Bogor palms planted in 1848. This means that they have a narrow genetic base.

TABLE 2
Selection Progress in Deli *Dura* Populations (Refs. 4,5)[a]

Progeny	Year	FFB yield (t/ha/y)	Oil/bunch (%)	Oil yield (t/ha/y)
F1 Bogor (unselected)	1878	17.9	17.3	3.1
Tj Morawa (unselected)	1885	16.6	18.5	3.1
Elmina (1st generation)	1933	21.3	17.2	3.7
OPRS (3rd–4th generations)	1969	26.9	18.4	5.0

[a]FFB, fresh fruit bunch.

TABLE 3
Yield Performance of Oil Palm Planting Materials (Refs. 5–7)[a]

Materials[b]	Year planted	Number of progenies	FFB (t/ha/y)	Oil/bunch (%)	Projected oil yield (t/ha/y)
DD × CI	1962	32	22.0	22.2	4.9
DD × UAC	1962	15	24.6	20.6	5.1
DD × SP	1962	6	21.1	23.0	4.9
DD × AVROS	1964	22	31.0	23.5	7.3
DD × AVROS	1968	16	31.1	22.1	6.9
DD × AVROS	1970	29	31.6	24.2	7.6
DD × AVROS	1979	5	34.5	25.8	8.9
DD × Dy-AVROS	1979	10	33.3	25.8	8.6
DD × Yangambi	1988	66	34.9	25.9	9.6

[a]See Table 2 for abbreviation.
[b]Progenies of crosses indicated in this column.

There seemed to be a low level of additive variation left in the Deli *dura* after several generations of selection, and most of the genetic variability present was nonadditive (8). Selection progress in subsequent generations will be low unless genetic variability is increased through crossing with other *dura* populations (6).

In view of the above problems, germplasm collections were made at its center of origin and diversity in Africa. These materials were evaluated, and selections were carried out in various populations for traits such as high yield, high IV, dwarfness, and pest, disease and abiotic stress resistance. One of the populations from the earlier prospected germplasm materials has been shown to be of great potential for future breeding and selection. This population gives oil yields ranging from 10.4 to 12.2 t/ha/y (Table 4). The selected elite materials have been distributed to the oil palm industry of Malaysia. This will not only broaden the genetic base of the breeding population but also increase the palm oil yield with high IV.

Lauric oils are a good source of feedstock for the oleochemical industry. With the decline in coconut oil (traditional source) production, palm kernel oil has replaced the former. It is profitable to have high kernel oil-producing palms, and there is much genetic variability within the *E. guineensis* population for large kernels (9).

A host of specialty oils can now be seriously considered, with the possibility of reduced development time by using the

TABLE 4
High-Yielding and Dwarf Nigerian *Tenera* Palms in PORIM[a]

Trial number	Family	Oil yield (kg/palm/y)	Oil yield (t/ha/y)	Height increment (cm/y)
0.149	28.17	83.3	12.2	23.1
0.149	19.11	75.9	11.2	21.5
0.149	13.05	76.3	11.3	24.0
0.150	16.21	70.4	10.4	24.9
0.150	19.13	71.6	10.6	22.5
Average commercial plantation			5.50	60.0

[a]PORIM, Palm Oil Research Institute of Malaysia.

TABLE 5
Fatty Acid Composition (%) of CPO from Nigerian Germplasm with High Iodine Value (IV)[a]

Palm number	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	IV
22	0.6	32.8	6.5	43.7	15.3	63.9
38	0.5	32.5	7.9	44.3	13.5	61.3
48	0.7	35.4	5.5	43.0	14.2	61.4
128	0.6	35.3	5.3	42.1	15.8	63.4
146	0.5	32.4	5.6	45.0	15.5	65.4
151	1.1	36.1	5.4	41.6	14.8	61.2
305	0.9	40.1	5.1	47.9	14.7	61.4
618	0.6	33.7	6.7	44.2	13.5	61.2
814	0.5	32.6	6.6	47.4	11.8	61.1
903	0.4	30.8	7.3	46.5	3.9	63.9
971	0.3	31.2	7.0	49.1	12.9	64.4
1861	1.4	37.0	6.4	36.2	17.6	61.4

[a]CPO, crude palm oil.

techniques of cloning, genetic engineering, and marker-assisted selection (10): (i) high-stearic acid oil—for production of cocoa butter substitute; (ii) high-carotene oil—for vitamin A and natural dye production, e.g., for use in instant noodles; (iii) high-tocopherol/tocotrienol oil—for vitamin E production; and (iv) industrial fatty acids, e.g., petroselenic acid, erucic acid, and ricinoleic acid for use in specialty plastics and lubricants production.

The fatty acid composition of palm oil, produced from current planting material, limits its share of the market for liquid and salad oils. Many Nigerian palms have oil (mesocarp) with IV above 60 (Table 5). With further fractionation of crude palm oil, the olein should attain an IV close to 70. With this IV, it would be possible to market palm olein as salad and liquid cooking oil in temperate countries. These palms have been crossed with the current breeding materials, and the IV of the progenies are comparable (Table 6) to the parental values, indicating that IV is highly inheritable (11).

Improvement of E. oleifera. Oil palm breeding efforts have been largely concentrated on *E. guineensis*. Improvements have been achieved as discussed above. However, the possibility of altering the fatty acid composition (Table 7), increasing the IV, slowing yearly height increment, and developing resistance to certain diseases has brought interest in *E. oleifera*. Although the commercial oil yield of *E. oleifera* is low, at less than 0.5 t/ha/y (Table 8), it has higher carotene (Table 9), vitamin E (tocopherols and tocotrienols) (Table 10), and sterol (Table 11) contents as compared to *E. guineensis* (12–14).

TABLE 6
Iodine Values of the Selected Nigerian Parents and Their Progenies (Ref. 11)

Parents	Iodine value	Progeny code	Iodine value (progeny means)
0.151/814	61.4	PK 486	61.42
0.151/1861	61.4	PK 591	61.86
0.151/971	64.4	PK 549	60.78
0.151/48	61.4	PK 515	64.17
0.151/618	61.2	PK 507	64.62
0.151/128	63.4	PK 540 (1403)	61.60
Commercial *tenera* planting material			52.00

TABLE 7
Fatty Acid Composition (%) of Oils from *Elaeis guineensis*, *E. oleifera*, and Their Hybrids (Refs. 12–14)

	E. guineensis (Eg)		*E. oleifera (Eo)*		*Eo* × *Eg* hybrid	
	PO[a]	PKO[a]	PO	PKO	PO	PKO
$C_{6:0}$	[b]	0.3	·	0.1	—	0.2
$C_{8:0}$		4.3	—	0.9	—	3.2
$C_{10:0}$	·	3.7	—	0.8		2.7
$C_{12:0}$	0.3	50.1	—	29.3		44.4
$C_{14:0}$	1.2	15.4	0.2	25.7	0.5	18.6
$C_{16:0}$	44.3	7.3	18.7	10.1	32.2	8.8
$C_{16:1}$	—	—	1.6	—	0.2	—
$C_{18:0}$	4.3	1.8	0.9	1.8	3.2	2.2
$C_{18:1}$	39.3	14.5	56.1	26.4	51.8	16.3
$C_{18:2}$	10.0	2.4	21.1	4.5	10.8	3.4
Others	0.6	0.2	1.0	0.4	0.9	0.2
Iodine value	55.0	18.2	85.0	31.5	67.5	21.0

[a]PO, palm oil; PKO, palm kernel oil.
[b]·, Not determined.

BIOENGINEERING

The fatty acid compositions (FAC) of palm oil and palm kernel oil of *E. guineensis* (Table 8) render these oils applicable to both edible and nonedible uses. However, to venture into new markets, a change in FAC is desirable. The interest in applying gene technology to the oil palm is thus for the production of oil with a high content of monounsaturated oleic acid. Such an oil has the potential to open up markets for palm oil in the liquid oil sector. In addition, oleic acid is useful as an industrial feedstock. A multidisciplinary approach has been taken at PORIM for the implementation of a concerted program to develop the tools and techniques required for genetic engineering of the oil palm (15). The strategy adopted is to alter the expression of genes of the enzymes that control the levels of palmitic and oleic acids in palm oil so that as much of the palmitate as possible is converted to oleate.

Biochemical studies, carried out on fatty acid synthesis in the oil palm mesocarp, indicated that the enzymes acyl carrier protein (ACP) thioesterase and β-keto acyl-acyl ACP synthase II control the levels of palmitate and oleate. Thioesterase activity in the crude extract showed a marked

TABLE 8
Yield of Pure *Elaeis oleifera*[a]

Progeny	FFB yield (t/ha/y)	Oil/bunch (%)	Oil yield (t/ha/y)
4.4-3	9.32	1.9	0.18
4.4-4	6.36	1.3	0.08
4.4-5	6.96	1.8	0.13
4.4-6	8.58	2.9	0.25
E. guineensis	25.31	25.8	6.55

[a]See Table 2 for abbreviation.

TABLE 9
Composition (%) of Carotenes of Palm Oils Derived from *Elaeis guineensis, E. oleifera,* and Their Hybrids (Ref. 12)

Type	*E. guineensis* (*Eg*)	*E. oleifera* (*Eo*)	*Eo* × *Eg* hybrid
Phytoene	1.27	1.12	1.83
Cis-β-carotene	0.68	0.48	0.38
Phytofluene	0.06	trace	trace
β-Carotene	56.02	54.08	60.53
α-Carotene	35.06	40.38	32.78
Cis-α-carotene	2.49	2.3	1.37
ζ-Carotene	0.69	0.36	1.13
γ-Carotene	0.33	0.08	0.23
δ-Carotene	0.83	0.09	0.24
Neurosporene	0.29	0.04	0.23
β-Zeacarotene	0.74	0.57	1.03
α-Zeacarotene	0.23	0.43	0.35
Lycopene	1.3	0.07	0.05
Total (ppm)	500–700	4300–4600	1250–1800

preference for palmitoyl ACP (16). Anion exchange chromatography resulted in the resolution of two peaks, one showing a marked preference for palmitoyl ACP and the other for oleoyl ACP. The results thus established that the chain termination reactions of $C_{16:0}$ and $C_{18:1}$ are independent, paving the way for the possibility of reducing palmitate by switching off the palmitoyl ACP thioesterase gene. At PORIM, the enzyme β-keto acyl ACP synthase II, or KASII, has been purified more than 10,000-fold. The activity of the enzyme in the mesocarp correlates positively with the level of unsaturation of the fatty acids present in the crude oil extract. Activity of KASII increased with fruit ripening, reaching a maximum at 20 wk after pollination. The results of these investigations suggest that high levels of palmitate in palm oil are likely to be due to low KASII activity and the specificity of the thioesterase for palmitoyl ACP. Because commercial palm oil is extracted from the mesocarp of the fruit, any effort at *in vitro* gene manipulation to change the oil will have to be directed to this tissue. For this purpose, PORIM's researchers have given much attention to the identification and isolation of genes that are specifically expressed in the mesocarp during oil synthesis. Because ACP activity has been shown to be induced just before the start of oil synthesis (17), the gene of this protein serves as a temporal marker. Attempts at isolating the ACP gene have resulted in the identification of several putative cDNA clones from a mesocarp library. Studies on differential gene regulation in the mesocarp employ the technique of comparing *in vitro* translated products of expressed genes during various stages of development. Stage-specific proteins have been identified (18). The techniques of differential hybridization and subtractive probe hybridization have been used to select several mesocarp-specific clones from a mesocarp library. Northern blot analysis has confirmed the tissue specificity of some of these clones.

TABLE 10
Composition (%) of Tocopherol and Tocotrienols of Palm Oils Derived from *Elaeis guineensis, E. oleifera,* and Their Hybrids (Ref. 12)

Type	*E. guineensis* (*Eg*)	*E. oleifera* (*Eo*)	*Eo* × *Eg* hybrid
α-Tocopherol	21	15	19
α-Tocotrienol	23	27	28
γ-Tocotrienol	45	54	42
δ-Tocotrienol	11	4	15
Total (ppm)	600–1000	700–1500	600–1600

TABLE 11
Composition (%) of Sterols of Palm Oils Derived from *Elaeis guineensis, E. oleifera,* and Their Hybrids (Ref. 12)

Type	*E. guineensis* (*Eg*)	*E. oleifera* (*Eo*)	*Eo* × *Eg* hybrid
β-Sitosterol	60	64	59
Campesterol	13	19	20
Stigmasterol	24	15	16
Cholesterol	3	2	5
Total (ppm)	250–620	3500–4000	1100–1250

In realizing the need for a means to introduce the manipulated gene(s) into the oil palm, much effort is being put into developing a reliable transformation method. Both direct DNA uptake and microprojectile bombardment have been attempted for this purpose. Transient expression of the β-glucuronidase (GUS) reporter gene has been observed in various transformed tissues, including pollens, young leaves, and embryogenic calli.

In conclusion, oil palm yields in *E.guineensis* have increased fourfold during the last 50 y (10). Breeding improvement contributed about 70% of the increase, and Deli *dura* had 65% increase during the same period. The switchover from *dura* to *tenera* contributed about 30% increase in yield. Progress of *tenera* improvement seemed to yield an increase of 9–12% per generation. IV also has increased from 52 to over 62 with the new breeding materials. Further yield and quality improvements are expected with new generations of planting materials, especially with the introduction of elite germplasm parents. *Elaeis oleifera* has low yields but is high in carotenes, vitamin E, and sterols and also has a high IV. Bioengineering, especially with the developments of transgenic plant technology, will assist in new cultivar development for novel oil compositions and specialty oils, in addition to attaining higher yields.

ACKNOWLEDGMENT

The authors thank the Director-General of PORIM for permission to present this paper.

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[Received May 30, 1997; accepted July 29, 1997]