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GARIS PANDUAN KEBANGSAAN UNTUK MENJALANKAN UJIAN KELAINAN, KESERAGAMAN DAN KESTABILAN

NATIONAL GUIDELINES FOR THE CONDUCT OF TESTS FOR DISTINCTNESS, UNIFORMITY AND STABILITY

KELAPA SAWIT
OIL PALM

Elaeis guineensis Jacq. dan terbitannya
Elaeis guineensis Jacq. and its derivatives

Nama Lain:
Alternative Names:

Nama Botani *Botanical Name*	Nama Tempatan *Local Name*	Nama Biasa *Common Name*
Elaeis guineensis Jacq.	Kelapa Sawit	Kelapa Sawit *Oil Palm*

JABATAN PERTANIAN MALAYSIA
DEPARTMENT OF AGRICULTURE MALAYSIA

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JADUAL KANDUNGAN
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1.0 PERKARA MENGENAI GARIS PANDUAN UJIAN
SUBJECT OF THESE TEST GUIDELINES

1.1 Garis panduan ini digunakan bagi semua varieti *Elaeis guineensis* Jacq. dan terbitannya.

These Test Guidelines apply to all varieties of Elaeis guineensis Jacq. and its derivatives.

1.2 Terbitan ini termasuklah hibrid antara spesies contohnya *Elaeis guineensis x Elaeis oleifera* dan/atau salingan dan hasil kacukbaliknya.

These derivatives include interspecific hybrids e.g. Elaeis guineensis x Elaeis oleifera and/or its reciprocals and their backcrosses.

2.0 BAHAN YANG DIPERLUKAN
MATERIAL REQUIRED

2.1. Pihak yang berwibawa (Lembaga Varieti Tumbuhan) memutuskan bilangan dan mutu bahan tanaman yang diperlukan bagi pengujian varieti serta masa dan tempat bahan tanaman itu perlu dihantar. Pemohon yang menyerahkan bahan dari Negara selain Malaysia di mana ujian dijalankan mestilah memastikan semua peraturan-peraturan kastam dan keperluan fitosanitari dipatuhi.

The competent authority (Plant Varieties Board) decides on the quantity and quality of the plant material required for testing the variety and when and where it is to be delivered. Applicants submitting material from a Country other than Malaysia in which the testing takes place must ensure that all customs formalities and phytosanitary requirements are complied with.

2.2 Varieti calon perlulah ditanam secara tempatan bagi tujuan pemeriksaan sebagai satu blok pemerhatian.

The candidate varieties must be grown locally for inspection as an observation block.

2.3. Bahan tanaman untuk diperiksa adalah varieti yang diterbitkan melalui biji benih dan/atau pembiakan tampang.

The materials to be examined shall either be seed derived and/or vegetatively propagated varieties.

2.4. Pokok yang terlibat untuk pemeriksaan hendaklah telah ditanam sekurang-kurangnya 8 tahun di ladang.

Age of palms to be inspected shall be at least 8 years after field planting.

2.5. Pemohon dikehendaki menyediakan sejumlah pokok minima dengan umur fisiologi yang sama (dalam satu blok penanaman) seperti berikut:

Applicant(s) shall provide a minimum number of palms of the same physiological age (in a growing block) as follows:

2.5.1 *Dura/Tenera* terbitan biji benih atau terbitannya : 30 pokok
Seed derived Dura/Tenera or its Derivatives :30 palms

2.5.2 *Pisifera* terbitan biji benih atau terbitannya : 5 pokok
Seed derived Pisifera or its Derivatives :5 palms

2.5.3 *Dura/Tenera/Pisifera* yang dibiak secara tampang atau terbitannya :10 pokok
Vegetatively propagated Dura/Tenera/Pisifera or its Derivatives :10 palms

2.6 Pihak yang berwibawa perlu membuat pemilihan secara rawak untuk pemeriksaan dan penilaian mengikut bilangan pokok seperti berikut:

The competent authority shall randomly pick the following number of palms for inspection and evaluation:

2.6.1 *Dura/Tenera* terbitan biji benih atau terbitannya : 10 pokok
Seed derived Dura/Tenera or its Derivatives : 10 palms

2.6.2 *Pisifera* terbitan biji benih atau terbitannya : 5 pokok
Seed derived Pisifera or its Derivatives : 5 palms

2.6.3 *Dura/Tenera/Pisifera* yang dibiak secara tampang atau terbitannya : 5 pokok
Vegetatively propagated Dura/Tenera/Pisifera or its Derivatives :5 palms

2.7. Bahan tanaman yang dibekalkan seharusnya tidak dijangkiti oleh mana-mana perosak atau penyakit yang utama semasa pemeriksaan.

The plant material(s) shall not be affected by any important pest or disease at the point of inspection.

2.8. Bahan tanaman tidak seharusnya melalui apa-apa rawatan yang mungkin menjejaskan ekspresi ciri varieti, melainkan dengan kebenaran atau permintaan pihak yang berwibawa. Sekiranya bahan tanaman telah dirawat, butir-butir penuh tentang rawatan mestilah diberikan.

The plant material should not have undergone any treatment, which would affect the expression of the characteristics of the variety, unless the competent authority allows or requests such treatment. If it has been treated, full details of the treatment must be given.

3.0 KAEDAH PEMERIKSAAN
METHOD OF INSPECTION

3.1. Tempoh Pemeriksaan
Period of Inspection

Pemeriksaan seharusnya dijalankan dalam tempoh 12 bulan. Pemeriksaan tambahan harus dijalankan jika dan apabila diputuskan perlu oleh pihak berwibawa.

Inspection shall be conducted within a period of 12 months. Additional inspection shall be conducted as and when deemed necessary by the competent authority.

3.2 Tempat Ujian
Location of Testing

Biasanya ujian dijalankan di satu tempat. Sekiranya wujud keperluan untuk membuat ujian di lebih dari satu tempat, panduan boleh diperolehi dari dokumen UPOV TGP/9: *Examining Distinctness*.

Tests are normally conducted at one place. In the case of tests conducted at more than one place, guidance is provided in UPOV document TGP/9: Examining Distinctness.

3.3. Keadaan-keadaan bagi Menjalankan Pemeriksaan
Conditions for Conducting the Examination

3.3.1 Bahan tanaman untuk pemeriksaan perlu berada dalam keadaan pertumbuhan yang biasa. Plot berasingan untuk pemerhatian dan pengukuran boleh digunakan tertakluk kepada budibicara pemeriksa.

The plant material(s) planted for inspection should be under conditions of normal growth. At the discretion of the examiners, separate plots for observation and measurement can be used.

3.3.2 Peringkat tumbesaran bagi penilaian
Stage of development for the assessment

Peringkat tumbesaran yang terbaik bagi penilaian setiap ciri ditunjukkan oleh huruf dalam lajur kedua Jadual Ciri. Peringkat tumbesaran yang ditandakan oleh setiap huruf dihuraikan dalam Bab 7.0.

The optimal stage of development for the assessment of each characteristic is indicated by a letter in the second column of the Table of Characteristics. The stages of development denoted by each letter are described at Chapter 7.0.

3.3.3 Jenis pemerhatian
Type of observation

Kaedah pemerhatian ciri yang disyorkan, diberikan oleh petunjuk berikut dalam lajur kedua Jadual Ciri, Bab 8.0 dokumen ini:

The recommended method of observing the characteristic is indicated by the following key in the second column of the Table of Characteristics in Chapter 8.0 of this document:

MG: satu pengukuran ke atas sekumpulan pokok atau bahagian-bahagian pokok
single measurement of a group of palms or parts of palms

MS: pengukuran beberapa pokok atau bahagian-bahagian pokok secara individu
measurement of a number of individual palms or parts of palms

VG: penilaian visual dengan satu pemerhatian ke atas sekumpulan pokok atau bahagian-bahagian pokok
visual assessment by a single observation of a group of palms or parts of palms

VS: penilaian visual dengan memerhati setiap pokok atau bahagian-bahagian pokok
visual assessment by observation of individual palms or parts of palms

3.4. Reka Bentuk Ujian
Test Design

Reka bentuk ujian di mana pokok atau bahagian-bahagian pokok boleh diasingkan untuk diukur atau dikira tanpa menjejaskan pemerhatian selanjutnya yang perlu dibuat sehingga tamat tempoh pemeriksaan.

The design of the tests should be such that palms or parts of palms may be removed for measurement or counting without prejudice to the observations which must be made up to the end of the inspection period.

3.5. Bilangan Pokok / Bahagian-bahagian Pokok untuk Diperiksa
Number of Palms / Parts of Palms to be Examined

Kecuali dinyatakan sebaliknya, semua pemerhatian pada pokok atau bahagian pokok yang diambil, berdasarkan kepada bilangan blok penanaman seperti yang dinyatakan dalam Seksyen 2.5 seperti berikut:

Unless otherwise indicated, all observations on palms or parts, based on number in growing block as per Section 2.5 shall be made as follows:

3.5.1 *Dura/Tenera* terbitan biji benih atau terbitannya : 10 pokok
Seed derived Dura/Tenera or its Derivatives : 10 palms

3.5.2 *Pisifera* terbitan biji benih atau terbitannya : 5 pokok
Seed derived Pisifera or its Derivatives : 5 palms

3.5.3 *Dura/Tenera/Pisifera* yang dibiak secara tampang atau terbitannya : 5 pokok
Vegetatively propagated Dura/Tenera/Pisifera or itsDerivatives : 5 palms

3.6. Ujian-ujian Tambahan
Additional Tests

Ujian-ujian tambahan untuk tujuan khusus boleh ditentukan.
Additional tests for specific purposes may be established.

3.7 Dokumen Sokongan
Supporting Documents

3.7.1 Pemohon adalah digalakkan untuk menyertakan sebarang dokumen sokongan kepada pihak yang berwibawa sebagai rujukan pada masa depan dalam kes penyelesaian pertikaian hak.

Applicant is encouraged to furnish supporting documents to the competent authority as future references in case of dispute settlement.

3.7.2 Dokumen sokongan seperti maklumat analisis tandan dan pengecapjarian DNA perlu mengikut tatacara yang disediakan oleh pihak yang berwibawa seperti di dalam Lampiran 1 dan Lampiran 2 masing-masing. Analisis tersebut seharusnya dilakukan oleh makmal atau makmal-makmal yang diiktiraf.

Supporting documents such as information on bunch analysis and DNA profiling shall follow the procedure as established by the competent authority in Annex 1 and Annex 2, respectively. The analyses should be made by an accredited laboratory or laboratories.

4.0 PENILAIAN KELAINAN, KESERAGAMAN DAN KESTABILAN
ASSESSMENT OF DISTINCTNESS, UNIFORMITY AND STABILITY

4.1. Kelainan
Distinctness

Sesuatu varieti adalah berlainan jika pada tarikh pemfailan permohonan, varieti baru tumbuhan itu dapat dibezakan dengan jelas daripada mana-mana varieti tumbuhan lain, yang kewujudannya telah diketahui umum.

A plant variety is distinct if on the date of filing of an application, the variety is clearly distinguishable from any other plant variety, the existence of which is a matter of common knowledge.

4.1.1 Syor-syor Umum
General Recommendations

Adalah penting bagi pengguna Garis Panduan Ujian ini untuk merujuk *dokumen UPOV TGP 1/3: General Introduction* sebelum membuat keputusan berkenaan dengan kelainan. Walau bagaimanapun, perkara berikut diberikan sebagai huraian lanjutan atau penekanan dalam Garis Panduan ini.

It is of particular importance for users of these Test Guidelines to consult the UPOV document TGP 1/3: General Introduction prior to making decisions regarding distinctness. However, the following points are provided for elaboration or emphasis in these Test Guidelines.

4.1.2 Perbezaan yang Konsisten
Consistent Differences

Sekiranya perbezaan yang diperhatikan antara varieti amat jelas, satu pemeriksaan adalah mencukupi. Sebagai tambahan, bagi sesetengah keadaan di mana perbezaan tidak jelas yang mungkin disebabkan oleh faktor persekitaran, lebih daripada satu pemeriksaan diperlukan untuk memastikan perbezaan yang diperhatikan antara

varieti adalah cukup konsisten.

The differences observed between varieties may be so clear that more than one inspection is not necessary. In addition, in some circumstances, the influence of the environment is not such that more than one inspection is required to provide assurance that the differences observed between varieties are sufficiently consistent.

4.1.3 Perbezaan Jelas
Clear Differences

Penentuan sama ada perbezaan antara dua varieti adalah jelas bergantung kepada banyak faktor, dan seharusnya mesti mengambil kira jenis ekspresi ciri yang diperiksa, iaitu sama ada ciri itu diekspresikan secara kualitatif, kuantitatif, atau pseudokualitatif. Maka, adalah penting bagi pengguna Garis Panduan Ujian ini untuk mengetahui syor-syor yang terkandung dalam *dokumen UPOV TGP 1/3: General Introduction* sebelum membuat keputusan berkenaan kelainan.

Determining whether a difference between two varieties is clear depends on many factors, and should consider, in particular, the type of expression of the characteristic being examined, i.e. whether it is expressed in a qualitative, quantitative, or pseudo-qualitative manner. Therefore, it is important that users of these Test Guidelines are familiar with the recommendations contained in the UPOV document TGP 1/3: General Introduction prior to making decisions regarding distinctness.

4.2 Keseragaman
Uniformity

Sesuatu varieti tumbuhan adalah seragam jika, tertakluk kepada perubahan yang dapat dijangka dari sifat tertentu pembiakannya, ianya turut seragam sepenuhnya dalam ciri-cirinya yang berkaitan/berkenaan.

A plant variety is uniform if, subject to the variation that may be expected from the particular features of its propagation, it is sufficiently uniform in its relevant characteristics.

4.2.1 Adalah penting bagi pengguna Garis Panduan Ujian ini untuk merujuk *dokumen UPOV TGP 1/3: General Introduction* sebelum membuat keputusan berkenaan dengan keseragaman. Walau bagaimanapun, perkara berikut diberikan sebagai huraian lanjutan atau penekanan dalam Garis Panduan ini.

It is of particular importance for users of these Test Guidelines to consult the UPOV document TGP 1/3: General Introduction prior to making decisions regarding uniformity. However, the following points are provided for elaboration or emphasis in these Test Guidelines.

4.2.2 Bagi penilaian keseragaman, bilangan jenis ganjil yang dibenarkan berdasarkan saiz sampel seperti yang dinyatakan dalam Seksyen 2.6 adalah seperti berikut:

For the assessment of uniformity, the number of off-types allowed, based on sample size as per Section 2.6 is as follows:

4.2.2.1 *Dura/Tenera* terbitan biji benih atau terbitannya :2 pokok
Seed derived Dura/Tenera or its Derivatives : 2 palms

4.2.2.2 *Pisifera* terbitan biji benih atau terbitannya :1 pokok
Seed derived Pisifera or its Derivatives : 1 palm

4.2.2.3 *Dura/Tenera/Pisifera* yang dibiak secara tampang atau terbitannya : 1 pokok
Vegetatively propagated Dura/Tenera/Pisiferaor its Derivatives :1 palm

4.3 Kestabilan
Stability

Sesuatu varieti adalah stabil jika ciri-cirinya yang berkaitan masih tidak berubah selepas pembiakan berulang kali atau, dalam hal suatu kitaran pembiakan yang tertentu, pada penghujung setiap kitaran tertentu itu.

A plant variety is stable if its relevant characteristics remain unchanged after repeated propagation or in the case of a particular cycle of propagation, at the end of each particular cycle.

4.3.1 Kebiasaannya, jarang dijalankan ujian kestabilan yang boleh menghasilkan kepastian seperti keputusan ujian kelainan dan keseragaman. Namun begitu, apabila sesuatu varieti terbukti seragam, ia dianggap stabil..

In practice, it is not usual to perform tests of stability that produce results as certain as those of the testing of distinctness and uniformity. However, when a variety has been shown to be uniform, it can also be considered to be stable.

5.0 PENGELOMPOKAN VARIETI
GROUPING OF VARIETIES

5.1 Varieti calon dibahagikan kepada kelompok bagi memudahkan penilaian kelainan, dibantu oleh penggunaan ciri pengelompokan.

Candidate varieties are divided into groups to facilitate the assessment of distinctness aided by the use of grouping characteristics.

5.2 Ciri pengelompokan ialah ciri yang keadaan ekspresinya telah didokumenkan walaupun dihasilkan di tempat berlainan. Ia boleh digunakan untuk memilih varieti yang diketahui umum yang boleh dibandingkan dengan varieti pemohon sama ada secara berasingan atau secara gabungan dengan ciri lain.

Grouping characteristics are those in which the documented states of expression, even where produced at different locations, can be used to select varieties of common knowledge. They can be compared to candidate varieties for examination of distinctness either individually or in combination with other such characteristics.

5.3 Berikut adalah ciri pengelompokan yang berguna berdasarkan Jadual Ciri di Bab 8.0:

The following are useful grouping characteristics based on Table of Characteristics in Chapter 8.0:

a) Rakis : panjang (ciri 5)
Rachis: length (characteristic 5)

b) Petiol: keratan rentas (ciri 7)
Petiole: cross section (characteristic 7)

c) Tandan : bentuk (ciri 13)
Bunch: shape (characteristic 13)

d) Buah: jenis (ciri 16)
Fruit: form (characteristic 16)

e) Buah : bentuk (ciri 17)
Fruit: shape (characteristic 17)

f) Buah: purata berat buah (ciri 19)
Fruit: Mean Fruit Weight (characteristic 19)

5.4 Garis panduan bagi penggunaan ciri pengelompokan, dalam proses pemeriksaan kelainan, diberi dalam *dokumen UPOV TGP 1/3: General Introduction*.

Guidance for the use of grouping characteristics, in the process of examining distinctness, is provided through the UPOV document TGP 1/3: General Introduction.

6.0 PENGENALAN KEPADA JADUAL CIRI
INTRODUCTION TO THE TABLE OF CHARACTERISTICS

6.1 Kumpulan Ciri-ciri
Categories of Characteristics

6.1.1 Ciri-ciri Garis Panduan Ujian Nasional
National Test Guidelines Characteristics

Ciri-ciri Garis Panduan Ujian Nasional ini dicadangkan oleh Pemeriksa-pemeriksa yang dilantik dan Pakar-pakar jemputan dan diluluskan oleh Lembaga Varieti Tumbuhan untuk pemeriksaan DUS.

National Test Guidelines characteristics are those which are proposed by appointed Examiners and Invited Experts and approved by the Plant Varieties Board for examination of DUS.

6.1.2 Ciri-ciri Bertanda Asterisk
Asterisked Characteristics

Ciri-ciri bertanda asterisk (ditandakan dengan *) yang termasuk dalam Garis Panduan Ujian merupakan ciri-ciri penting bagi penyelarasan pencirian varieti pada peringkat antarabangsa dan seharusnya selalu diperiksa untuk DUS, dan dimasukkan dalam pencirian varieti, kecuali apabila keadaan ekspresi ciri yang sebelumnya atau keadaan persekitaran kawasan menjadikannya tidak sesuai.

*Asterisked characteristics (denoted by *) are those included in the Test Guidelines which are important for the international harmonization of variety descriptions and should always be examined for DUS and included in the variety description, except when the state of expression of a preceding characteristic or regional environmental conditions render this inappropriate.*

6.2 Tahap Ekspresi dan Catatan Berkaitan
States of Expression and Corresponding Notes

Tahap ekspresi diberi bagi setiap ciri untuk menjelaskan ciri dan untuk menyelaraskan pencirian. Setiap tahap ekspresi diperuntukkan catatan berangka yang sepadan bagi memudahkan merekod data dan bagi penerbitan serta pertukaran pencirian.

States of expression are given for each characteristic to define the characteristic and to harmonize descriptions. Each state of expression is allocated a corresponding numerical note for ease of recording of data and for the production and exchange of the description.

6.3 Jenis Ekspresi
Types of Expression

Penjelasan tentang jenis ekspresi ciri (kualitatif, kuantitatif dan pseudokualitatif) diberi dalam *dokumen UPOV TGP 1/3: General Introduction*.

An explanation of the types of expression of characteristics (qualitative, quantitative and pseudo-qualitative) is provided in the UPOV document TGP 1/3: General Introduction.

6.4 Varieti-varieti Contoh
Example Varieties

Di mana sesuai, varieti-varieti contoh diberi untuk menjelaskan keadaan ekspresi setiap ciri.

Where appropriate, example varieties are provided to clarify the states of expression of each characteristic.

6.5 Petunjuk
Legend

(*) Ciri bertanda asterisk – lihat Bab 6.1.2
Asterisked characteristic – see Chapter 6.1.2

QL Ciri kualitatif – lihat Bab 6.3
Qualitative characteristic – see Chapter 6.3
QN Ciri kuantitatif – lihat Bab 6.3
Quantitative characteristic – see Chapter 6.3
PQ Ciri pseudokualitatif – lihat Bab 6.3
Pseudo-Qualitative characteristic – see Chapter 6.3

MG: lihat bahagian 3.3.3
see section 3.3.3
MS: lihat bahagian 3.3.3
see section 3.3.3
VG: lihat bahagian 3.3.3
see section 3.3.3
VS: lihat bahagian 3.3.3
see section 3.3.3

(a) – (c) Lihat Penjelasan meliputi beberapa ciri dalam Bab 7.0.
See Explanations Covering Several Characteristics in Chapter 7.0.

(+) Lihat Penjelasan bagi ciri individu dalam Bab 8.0.
See Explanations for Individual Characteristics in Chapter 8.0.

7.0 PENJELASAN JADUAL CIRI
EXPLANATIONS ON THE TABLE OF CHARACTERISTICS

Penjelasan meliputi beberapa ciri
Explanations covering several characteristics

Ciri-ciri yang mengandungi petunjuk berikut dalam lajur kedua Jadual Ciri seharusnya diperiksa seperti yang ditunjukkan di bawah:

Characteristics containing the following key in the second column of the Table of Characteristics should be examined as indicated below:

(a) Pokok dan pelepah: Semua pengukuran dan pemerhatian pada pokok dan pelepah perlu dilakukan sekurang-kurangnya 8 tahun selepas penanaman di ladang.

Palm and frond: All measurements and observation on the tree and the frond should be made on at least 8 year old plants after field planting.

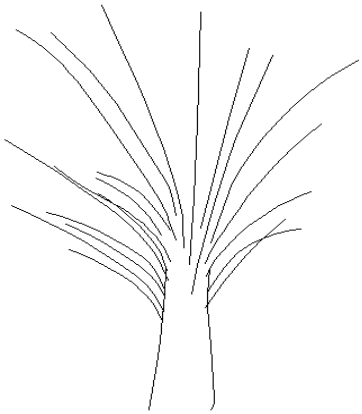
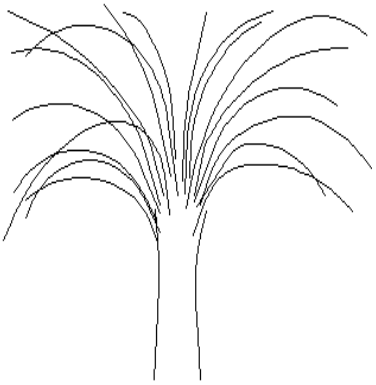
(b) Tandan: Semua pengukuran dan pemerhatian pada tandan perlu dibuat pada tandan baru dituai serentak dengan pengukuran pokok dan pelepah. Pemeriksaan mesti dilakukan sekurang-kurangnya 2 kali dalam tempoh setahun.

Bunch: All measurements and observation on the bunch should be made on the fresh bunches at the same time as the measurement of the palm and the frond. Inspection shall be made at least twice over a period of one year.

(c) Buah: Semua pengukuran dan pemerhatian pada buah seharusnya dibuat pada sampel yang diambil daripada lima (5) tandan masak yang baru dituai. Sepuluh (10) buah luaran masak yang terletak di bahagian atas diambil dari setiap tandan. Sekiranya perlu, pemeriksaan boleh diulangi untuk kitaran penuaian seterusnya.

Fruit: All measurements and observation on the fruit should be made on the samples which are taken from five (5) newly harvested ripe bunches. Ten (10) ripe outer fruits are taken from the top of each bunch. If necessary, inspection can be repeated for more than one harvesting rounds.

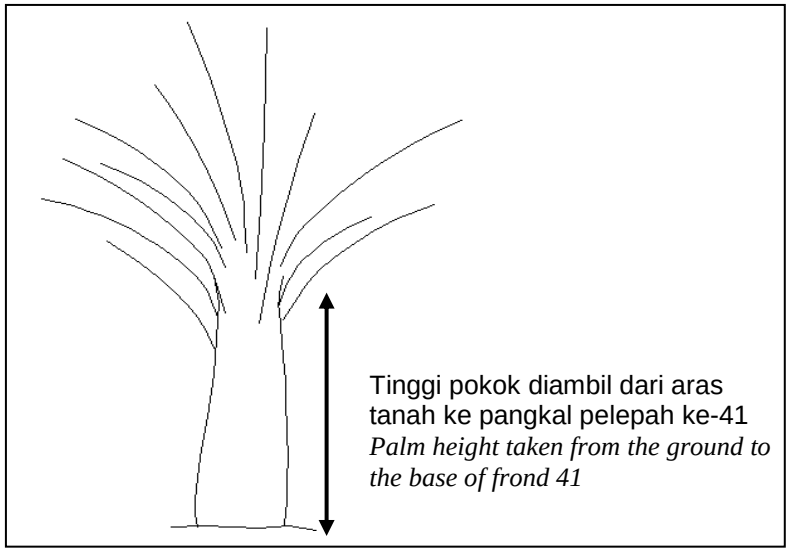
8.0 JADUAL CIRI
TABLE OF CHARACTERISTICS

BIL. *NO.*		**CIRI** *CHARACTERISTIC*	**KEADAAN** *STATE*	**VARIETI CONTOH** *EXAMPLE VARIETIES*	**NOTA** *NOTE*
1. (+) QL	VG (a)	Pokok: reka bentuk *Palm: architecture*	menegak *erect* terbuka *open*		1 2
Tamb. (1): Pokok: reka bentuk *Ad. (1): Palm: architecture*  1 menegak *erect*  2 terbuka *open*					
2. (*) (+) QN	MS (a)	Pokok: tinggi *Palm: height*	rendah *low* sederhana *medium* tinggi *high*		3 5 7

BIL. *NO.*		CIRI *CHARACTERISTIC*	KEADAAN *STATE*	VARIETI CONTOH *EXAMPLE VARIETIES*	NOTA *NOTE*

Tamb. (2): Pokok: tinggi
Ad. (2): Palm:height

Tinggi pokok diukur dari aras tanah ke pangkal pelepah ke-41 (pelepah ke-6 pada *parastichy* pertama)
Palm height is measured from ground level to the base of frond 41 (6th frond on 1st parastichy).



3. (+) QN	MS (a)	Pokok: kadar peningkatan ketinggian *Palm: height increment*	rendah *low*		3
			sederhana *medium*		5
			tinggi *high*		7

Tamb. (3): Pokok: kadar peningkatan ketinggian
Ad. (3): Palm: height increment

Kadar peningkatan ketinggian pokok diukur dengan membahagi tinggi pokok(Ht) dengan umur pokok selepas penanaman di ladang (t) tolak 2 seperti berikut:

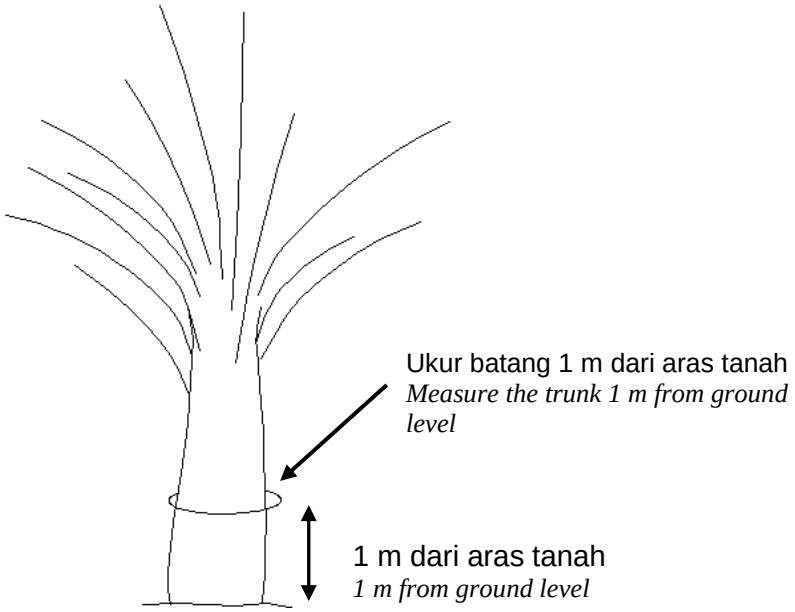
Palm height increment is measured by dividing palm height (Ht) palm age (t) after field planting minus 2 as follows:

Kadar Peningkatan ketinggian Pokok
Palm height increment $= \frac{Ht\ (cm)}{t - 2}$

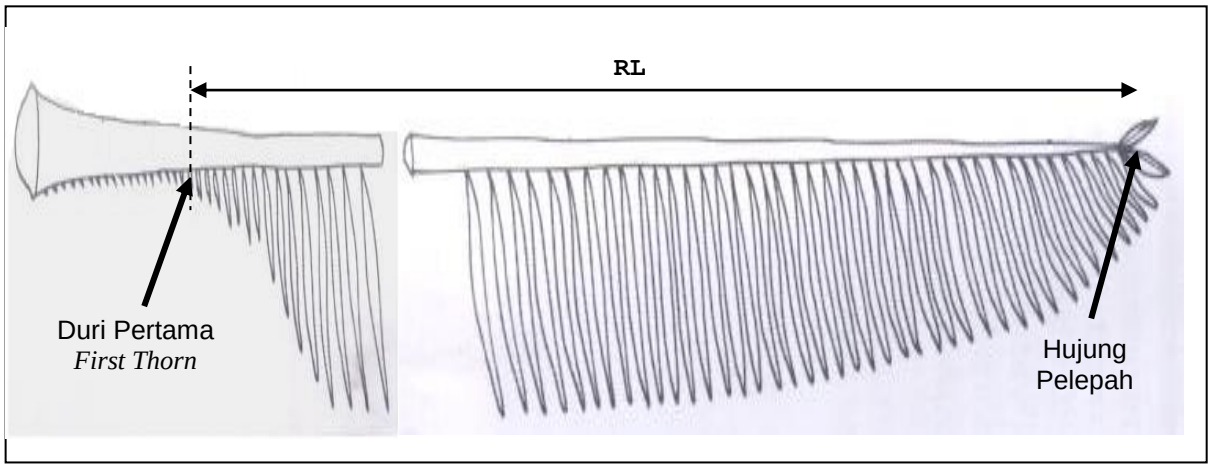
BIL. *NO.*		CIRI *CHARACTERISTIC*	KEADAAN *STATE*	VARIETI CONTOH *EXAMPLE VARIETIES*	NOTA *NOTE*
4. (+) QN	MS (a)	Pokok: lilitan batang *Palm: trunk girth*	kecil *small* sederhana *medium* besar *large*		3 5 7

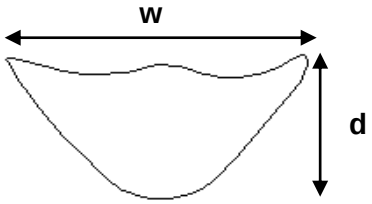
Tamb.(4): Pokok: lilitan batang
Ad. (4): Palm: trunk girth

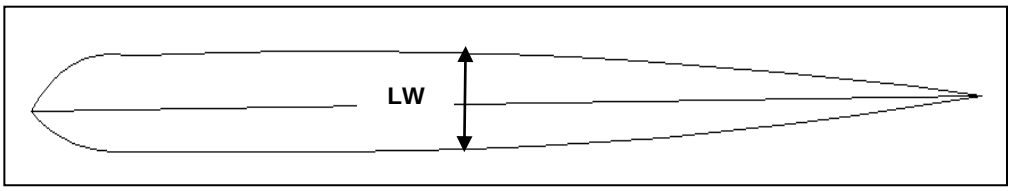
Lilitan batang diukur pada ketinggian 1 meter dari aras tanah dengan menggunakan angkup batang.
Trunk girth is measured at 1 meter above the ground level using trunk calipers.



5. (*) (+) QN	MS (a)	Rakis: panjang *Rachis: length (RL)*	pendek *short* sederhana *medium* panjang *long*		3 5 7

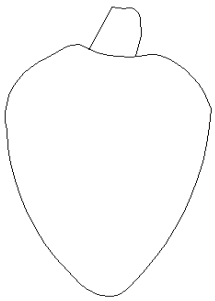
BIL. *NO.*		CIRI *CHARACTERISTIC*	KEADAAN *STATE*	VARIETI CONTOH *EXAMPLE VARIETIES*	NOTA *NOTE*
		Tamb. (5): Rakis: panjang (RL) *Ad. (5): Rachis: length (RL)* Rakis perlu disampel dari pelepah ke-17 semasa pemeriksaan yang terletak pada pelepah ke-3 sepanjang *parastichy* pertama. Ukuran dari duri pertama pada petiol ke hujung pelepah adalah panjang rakis. *Rachis is to be sampled from frond 17 during inspection which is located on the 3rd frond along the 1st parastichy. Measurement from the first thorn of the petiole to the frond tip is indicated as rachis length.*  			
6. PQ	VS (a)	Rakis: warna *Rachis: colour*	kuning *yellow* hijau kekuningan *yellowish green* hijau pudar *pale green* hijau *green* hijau gelap *dark green*		1 2 3 4 5
7. (*) (+) QN	MG (a)	Petiol: keratan rentas (PCS) *Petiole: cross section (PCS)*	kecil *small* sederhana *medium* besar *large*		3 5 7

BIL. *NO.*		CIRI *CHARACTERISTIC*	KEADAAN *STATE*	VARIETI CONTOH *EXAMPLE VARIETIES*	NOTA *NOTE*
Tamb. (7): Petiol: keratan rentas (PCS) *Ad. (7): Petiole: cross section (PCS)* Lebar dan kedalaman petiol diukur pada duri pertama pelepah ke-17 dan PCS dapat dihitung dengan menggunakan formula berikut: *Width and depth of the petiole is measured at the first thorn from frond 17 and PCS will be estimated using formulae as follows;* PCS = Lebar Petiol (w) x Kedalaman Petiol (d) *PCS = Petiole width (w) x Petiole depth (d)*  					
8. PQ	VS (a)	Petiol: warna *Petiole: colour*	kuning *yellow* hijau kekuningan *yellowish green* hijau *green* hijau gelap *dark green*		1 2 3 4
9. (*) (+) QN	MG (a)	Lai daun: panjang *Leaflet: length*	pendek *short* sederhana *medium* panjang *long*		3 5 7

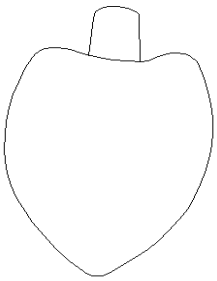
BIL. *NO.*		CIRI *CHARACTERISTIC*	KEADAAN *STATE*	VARIETI CONTOH *EXAMPLE VARIETIES*	NOTA *NOTE*
Tamb. (9): Lai daun: panjang *Ad. (9): Leaflet: length* Panjang lai daun diukur dari pangkal ke hujung lai daun. Tiga lai daun dari dua belah rakis paling dekat dengan ligul pelepah adalah disampel dari pelepah ke-17 semasa pemeriksaan. *Leaflet length is measured from the base to the tip of the leaflet. Three leaflets from each side of the rachis closest to the frond ligules are to be sampled from frond 17 during inspection.*  Panjang *Length*					
10. (*) (+) QN	MG (a)	Lai daun : lebar *Leaflet: width*	sempit *narrow* sederhana *medium* lebar *broad*		3 5 7
Tamb. (10): Lai daun: lebar *Ad. (10): Leaflet: width* Dari sampel Ciri 9, lebar lai daun diukur pada titik di mana lai daun yang dilipat adalah sama panjang. *From Char. 9 samples, leaflet width is measured at the point where the leaflet is folded at equal length.*  					
11. (*) QN	MG (a)	Lai daun: bilangan *Leaflet: number*	sedikit *few* sederhana *medium* banyak *many*		3 5 7

BIL. *NO.*		CIRI *CHARACTERISTIC*	KEADAAN *STATE*	VARIETI CONTOH *EXAMPLE VARIETIES*	NOTA *NOTE*
12. QN	MG (a)	Lai daun: susunan *Leaflet: arrangement*	paksi tunggal *single plane* paksi dua *double plane*		1 2
13. (+) PQ	VS (b)	Tandan: bentuk *Bunch: shape*	obovat *obovate* bentuk jantung *heart-shape* bulat *globular*		1 2 3

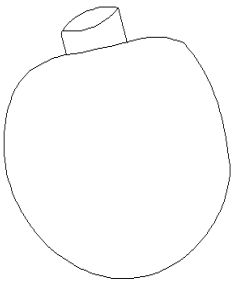
Tamb. (13): Tandan: bentuk
Ad. (13): Bunch: shape



1
obovat
obovate

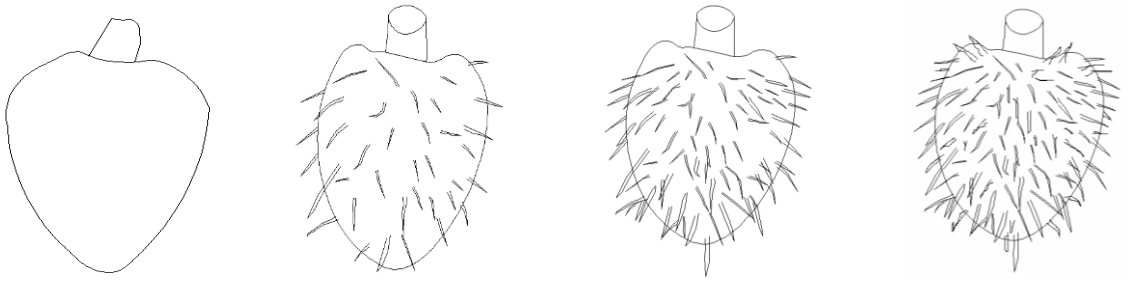


2
bentuk jantung
heart-shape



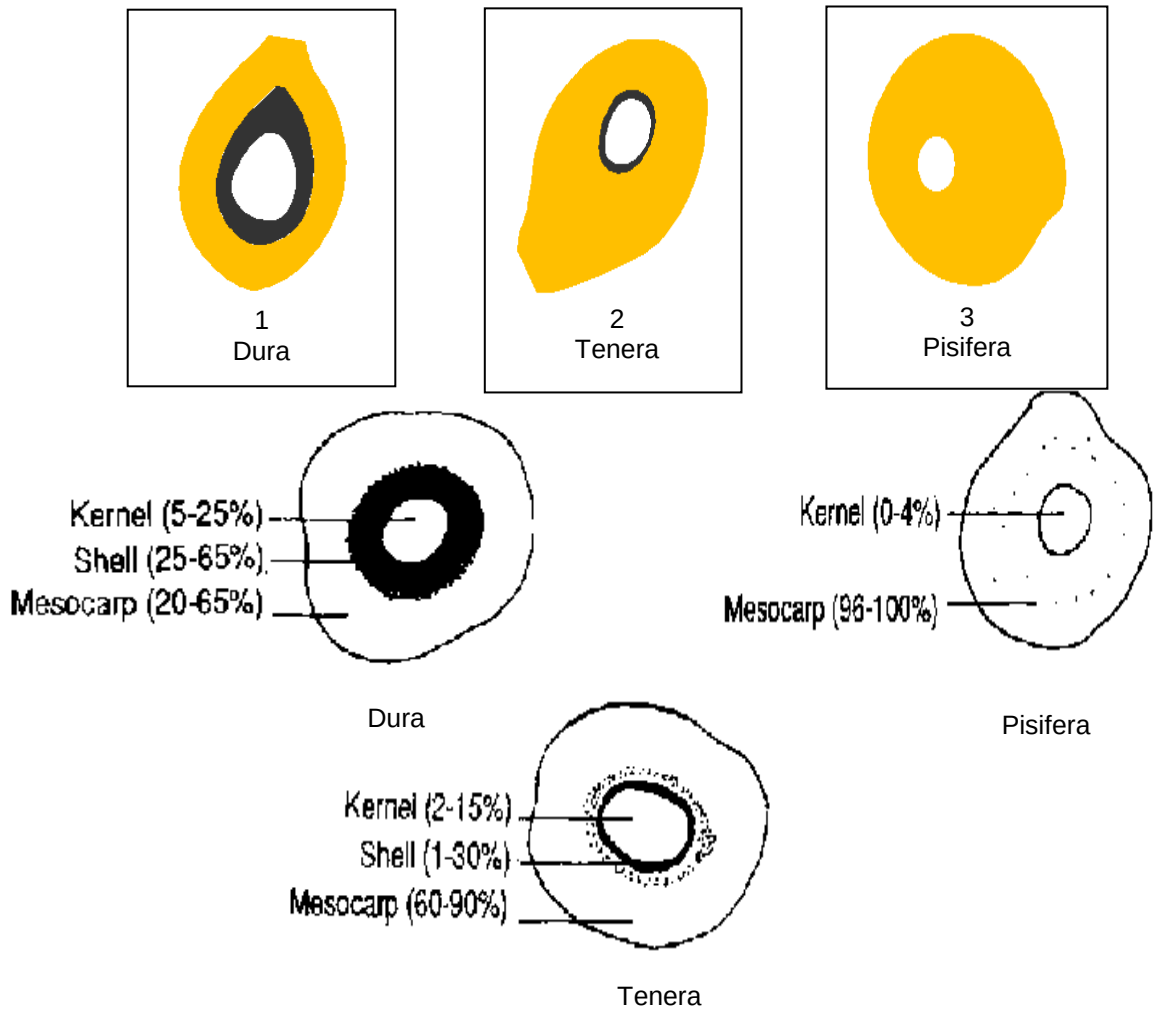
3
bulat
globular

14. (+) QN	VS (b)	Tandan: kepadatan duri *Bunch: density of spines*	tiada *none* jarang *sparse* sederhana *medium* padat *dense*		1 3 5 7

BIL. *NO.*		CIRI *CHARACTERISTIC*	KEADAAN *STATE*	VARIETI CONTOH *EXAMPLE VARIETIES*	NOTA *NOTE*
Tamb. (14): Tandan: kepadatan duri *Ad. (14): Bunch: density of spines*  1 tiada *none* 3 jarang *sparse* 5 sederhana *medium* 7 padat *dense*					
15. QN	MG (b)	Tandan: nisbah tangkai kepada tandan *Bunch: stalk to bunch ratio (% by weight)*	rendah *low* sederhana *medium* tinggi *high*		3 5 7
16. (*) (+) QL	VS (c)	Buah: jenis *Fruit: form*	dura *dura* tenera *tenera* pisifera *pisifera*		1 2 3

BIL. *NO.*		CIRI *CHARACTERISTIC*	KEADAAN *STATE*	VARIETI CONTOH *EXAMPLE VARIETIES*	NOTA *NOTE*

Tamb. (16): Buah: jenis
Ad. (16): Fruit: form

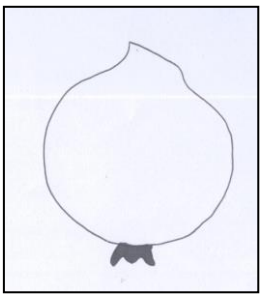


Gambar rajah : Buah Dura, Tenera dan Pisifera
Illustration: Dura, Tenera and Pisifera Fruit

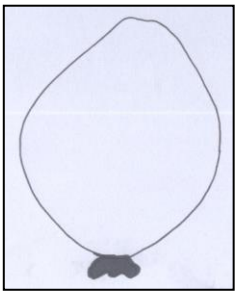
BIL. *NO.*		CIRI *CHARACTERISTIC*	KEADAAN *STATE*	VARIETI CONTOH *EXAMPLE VARIETIES*	NOTA *NOTE*
17. (+) PQ	VS (c)	Buah: bentuk *Fruit: shape*	bulat *circular* elips *elliptic* rombik *rhombic* ovate *ovate* obovat *obovate* lonjong *oblong*		1 2 3 4 5 6

Tamb. (17): Buah: bentuk
(sampel rawak daripada sepuluh buah luaran yang masak diambil dari setiap tandan baru dituai seperti dinyatakan dalam Seksyen 2.5.)

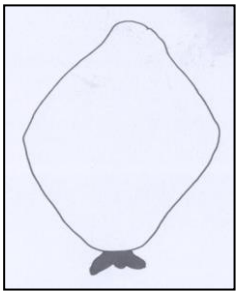
Ad. (17): Fruit: shape
(random sample of ten ripe outer fruits are taken from each newly harvested ripe bunch as per Section 2.5.)



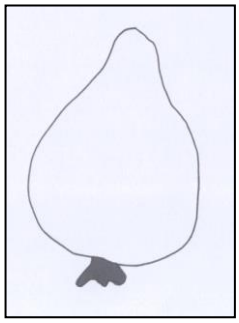
1
bulat
circular



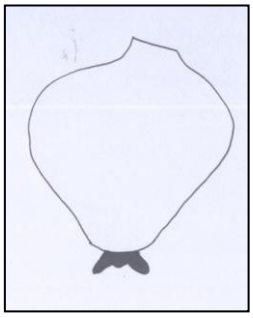
2
elips
elliptic



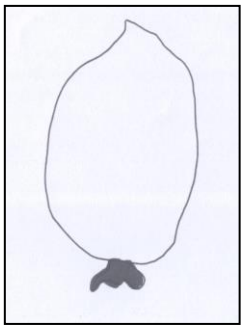
3
rombik
rhombic



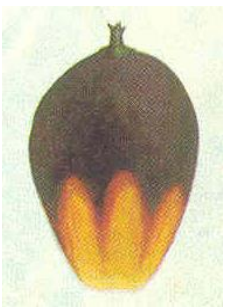
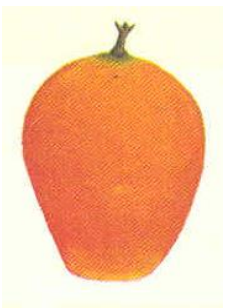
4
ovat
ovate



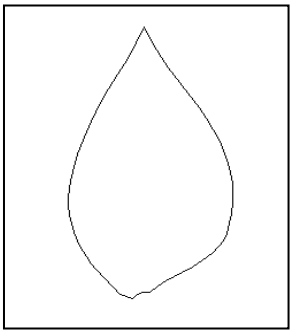
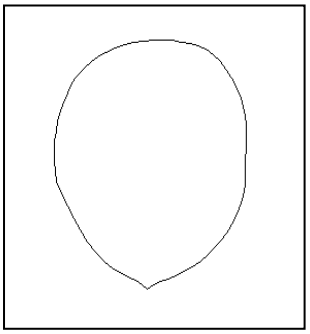
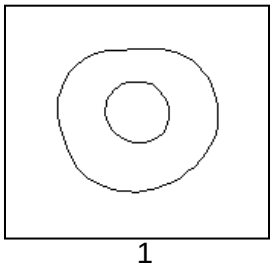
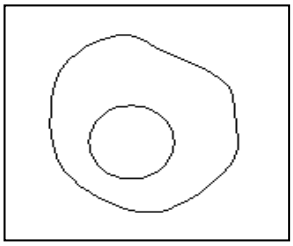
5
obovat
obovate



6
lonjong
oblong

BIL. *NO.*		CIRI *CHARACTERISTIC*	KEADAAN *STATE*	VARIETI CONTOH *EXAMPLE VARIETIES*	NOTA *NOTE*
18. (*) (+) QL	VS (c)	Buah: warna *Fruit: colour*	albescens *albescens* nigrescens *nigrescens* virescens *virescens*		1 2 3
Tamb. (18): Buah: warna *Ad. (18): Fruit: colour*    1 albescens *albescens* 2 nigrescens *nigrescens* 3 virescens *virescens*					
19. QN	MG (c)	Buah: Purata Berat Buah (MFW) *Fruit: Mean Fruit Weight (MFW)*	rendah *low* sederhana *medium* tinggi *high*		3 5 7
20. QN	MG (c)	Buah: nisbah isirong kepada buah (% mengikut berat) *Fruit: kernel to fruit ratio (% by weight)*	rendah *low* sederhana *medium* tinggi *high*		3 5 7

BIL. *NO.*		**CIRI** *CHARACTERISTIC*	**KEADAAN** *STATE*	**VARIETI CONTOH** *EXAMPLE VARIETIES*	**NOTA** *NOTE*
21. (*) QN	MG (c)	Buah: nisbah tempurung kepada buah (% mengikut berat) *Fruit: shell to fruit ratio (% by weight)*	rendah *low* sederhana *medium* tinggi *high*		3 5 7
22. QN	MG (c)	Buah: nisbah mesokarpa kepada buah (5 mengikut berat) *Fruit: mesocarp to fruit ratio(% by weight)*	rendah *low* sederhana *medium* tinggi *high*		3 5 7
23. (*) QL	VS (c)	Mesokarpa: warna *Mesocarp: colour*	kuning muda *light yellow* kuning *yellow* jingga muda *light orange* jingga *orange* jingga tua *dark orange*		1 2 3 4 5
24. (*) (+) QL	VS (c)	Biji: bentuk *Nut: shape*	tajam *pointed* bulat *rounded*		1 2

BIL. *NO.*		CIRI *CHARACTERISTIC*	KEADAAN *STATE*	VARIETI CONTOH *EXAMPLE VARIETIES*	NOTA *NOTE*
Tamb. (24): Biji: bentuk *Ad. (24): Nut: shape*  1 tajam *pointed*  2 bulat *rounded*					
25. (*) (+) QL	VS (c)	Biji: kedudukan isirong (keratan rentas) *Nut: kernel position (cross-section)*	memusat *center* luar pusat *off-center*		1 2
Tamb. (25): Biji: kedudukan isirong (keratan rentas) *Ad. (25): Nut: kernel position (cross-section)*  1 memusat *center*  2 luar pusat *off-center*					
26. QN	MG (c)	Biji: diameter *Nut: diameter*	kecil *small* sederhana *medium* besar *large*		*3* *5* *7*

9.0 RUJUKAN
LITERATURE

C.W.S. Hartley (1967). Tropical Agriculture Series. *The Oil Palm (Elaeis guineensis* Jacq.*)*. London: Longman and Green. 806p.

R. H. V. Corley and P.H.B. Tinker (2003). *The Oil Palm* (4th Edition). Blackwell Publishing, Incorporated. 592p.

T. H. Fairhurst and Rolf Härdter (2003). *Oil Palm: Management for Large and SustainableYields.* Potash & Phospahte Institute and International Potash Institute. First Edition 2003, pp. 13-26.

10.0 SOALSELIDIK TEKNIKAL
TECHNICAL QUESTIONNAIRE

BORANG SOALSELIDIK TEKNIKAL
TECHNICAL QUESTIONNAIRE

untuk dilengkapi berkaitan dengan permohonan Hak Pembiakbaka Tumbuhan
to be completed in connection with an application for Plant Breeders' Rights

UNTUK KEGUNAAN RASMI
FOR OFFICIAL USE

Nombor Rujukan : ***Reference Number***	**Tarikh Permohonan** : *Application date* **(tidak boleh diisi oleh pemohon)** (*not to be filled in by the applicant*)

1. BAHAN UJIAN
SUBJECT OF THE TECHNICAL QUESTIONNAIRE

1.1 Nama Botani : Elaeis guineensis Jacq.
Botanical Name

1.2 Nama Biasa : KELAPA SAWIT
Common name OIL PALM

1.3 Nama Tempatan : KELAPA SAWIT
Local name

2. PEMOHON
APPLICANT

Nama Pemohon : _____
Applicant Name

Alamat : _____
Address _____
_____

No. Telefon : _____
Telephone No.

No. Faks : _____
Fax No.

Alamat e-mel : _____
E-mail address

Pembiakbaka : _____
Breeder
(jika berlainan daripada pemohon)
(if different from applicant)

3. NAMA YANG DICADANGKAN DAN RUJUKAN PEMBIAKBAKA
PROPOSED DENOMINATION AND BREEDER'S REFERENCE

Nama yang dicadangkan _____ _____ _____
Proposed denomination **pilihan pertama** (*1st choice*) **pilihan kedua** (*2nd choice*) **pilihan ketiga** (*3rd choice*)

Rujukan pembiakbaka _____
Breeder's reference

4. MAKLUMAT SKIM PEMBIAKBAKAAN DAN PEMBIAKAN VARIETI
INFORMATION ON THE BREEDING SCHEME AND PROPAGATION OF THE VARIETY

4.1 Skim pembiakbakaan :
Breeding scheme

Varieti terhasil daripada :
Variety resulting from

☐ Kacukan terkawal (sila nyatakan varieti induk)
Controlled cross (please state parent varieties)

☐ Mutasi (sila nyatakan varieti induk)
Mutation (please state parent varieties)

☐ Penemuan dan pembangunan (sila nyatakan di mana dan bila ditemui dan bagaimana dibangunkan)
Discovery and development (please state where and when discovered and how developed)

☐ Lain-lain (sila berikan butir-butir)
Others (please provide details)

4.2 Kaedah pembiakan varieti
Method of propagating the variety

4.2.1 Pembiakan tampang
Vegetative propagation

☐ pembiakan *in vitro*
in vitro propagation

☐ lain-lain (nyatakan kaedah)
others (state method)

4.2.2 ☐ Biji benih
Seed

4.2.3 ☐ Lain-lain (sila berikan butir-butir)
Others (please provide details)

Dalam kes varieti hibrid, skim pengeluaran hibrid perlu disediakan pada helaian berasingan. Butir-butir semua baris induk yang diperlukan untuk pembiakan hibrid berkenaan juga perlu disediakan, contohnya:

In the case of hybrid varieties the production scheme for the hybrid should be provided on a separate sheet. Details of all the parental lines required for propagating the hybrid should also be provided e.g.

Hibrid Sehala
Single Hybrid

(… induk betina...) x (... induk jantan...)
(… female parent …) x (… male parent …)

Hibrid Tiga Hala
Three-Way Hybrid

(... baris betina ...) x (... baris jantan ...)
→ hibrid sehala digunakan sebagai induk betina x (... induk jantan...)

(… female line …) x (… male line …)
→ single hybrid used as female parent x (… male parent …)

dan perlu dikenalpasti terutamanya:
and should identify in particular:

(a) mana-mana baris mandul jantan
any male sterile lines

(b) sistem penyelenggaraan baris mandul jantan
maintenance system of male sterile lines

 # Pihak berkuasa mungkin membenarkan maklumat tertentu diberi dalam bahagian sulit Borang Soalselidik Teknikal.
The authority may allow certain information to be provided in a confidential section of the Technical Questionnaire.

5. CIRI-CIRI VARIETI YANG PERLU DINYATAKAN

CHARACTERISTICS OF THE VARIETY TO BE INDICATED

Nombor dalam kurungan merujuk kepada ciri yang sepadan dengan Garis Panduan Ujian; sila tandakan catatan yang paling sepadan.

The number in brackets refers to the corresponding characteristic in Test Guidelines; please mark the note which best corresponds.

Bil. No.	**Ciri** *Characteristic*	**Keadaan** *State*	**Varieti Contoh** *Example varieties*	**Catatan** *Note*
5.1 (5)	Rakis: panjang *Rachis: length (RL)*	pendek *short*		3
		sederhana *medium*		5
		panjang *long*		7
5.2 (7)	Petiol: keratan rentas (PCS) *Petiole: cross section (PCS)*	kecil *small*		3
		sederhana *medium*		5
		besar *large*		7
5.3 (13)	Tandan: bentuk *Bunch: shape*	obovat *obovate*		1
		bentuk jantung *heart-shape*		2
		bulat *globular*		3
5.4 (16)	Buah: jenis *Fruit: form*	dura *dura*		1
		tenera *tenera*		2
		pisifera *pisifera*		3

Bil. No.	**Ciri** *Characteristic*	**Keadaan** *State*	**Varieti Contoh** *Example varieties*	**Catatan** *Note*
5.5 (17)	Buah: bentuk *Fruit: shape*	bulat *circular*		1
		elips *elliptic*		2
		rombik *rhombic*		3
		ovate *ovate*		4
		obovat *obovate*		5
		lonjong *oblong*		6
5.6 (19)	Buah: Purata Berat Buah (MFW) *Fruit: Mean Fruit Weight (MFW)*	rendah *low*		3
		sederhana *medium*		5
		tinggi *high*		7

6. VARIETI SERUPA DAN PERBEZAAN DARIPADA VARIETI CALON

SIMILAR VARIETIES AND DIFFERENCES FROM THESE VARIETIES

Sila gunakan jadual dan kotak berikut untuk mengulas bagi memberikan maklumat berkenaan perbezaan antara varieti calon dengan varieti (atau varieti-varieti) yang paling serupa mengikut pengetahuan anda. . Maklumat ini boleh membantu pihak berwibawauntuk menjalankan pemeriksaan kelainan dengan lebih cekap.

Please use the following table and box for comments to provide information on how your candidate variety differs from the variety (or varieties) which, to the best of your knowledge, is (or are) most similar. This information may help the examination authority to conduct its examination of distinctness in a more efficient way.

Nama varieti (varieti-varieti) yang serupa dengan varieti calon *Denomination(s) of variety(ies) similar to your candidate variety*	**Ciri (ciri-ciri) varieti calon yang berbeza daripada varieti (varieti-varieti) serupa** *Characteristic(s) in which your candidate variety differs from the similar variety(ies)*	**Terangkan ekspresi ciri (ciri-ciri) bagi varieti (varieti-varieti) serupa** *Describe the expression of the characteristic(s) for the similar variety(ies)*	**Terangkan ekspresi ciri (ciri-ciri) bagi varieti calon** *Describe the expression of the characteristic(s) for your candidate variety*
[Contoh] *[Example]*	**[Tandan: bentuk]** *[Bunch: shape]*	**[ovat]** *[ovate]*	**[obovat]** *[obovate]*

Ulasan :
Comments

7. MAKLUMAT TAMBAHAN YANG BOLEH MEMBANTU DALAM PEMERIKSAAN VARIETI

ADDITIONAL INFORMATION WHICH MAY HELP IN THE EXAMINATION OF THE VARIETY

7.1 Sebagai tambahan kepada maklumat yang diberi dalam bahagian 5 dan 6, adakah apa-apa ciri tambahan yang boleh membantu untuk membezakan varieti?

In addition to the information provided in sections 5 and 6, are there any additional characteristics which may help to distinguish the variety?

☐ Ada *Yes* ☐ Tiada *No*

(Jika ada, berikan butir-butir) *(If yes, please provide details)*

7.2 Adakah apa-apa keadaan khusus bagi menanam varieti atau menjalankan pemeriksaan?

Are there any specific conditions for growing the variety or conducting the examination?

☐ Ada *Yes* ☐ Tiada *No*

(Jika ada, berikan butir-butir) *(If yes, please provide details)*

7.3 Maklumat lain

Other information

7.4 Gambar berwarna yang mewakili varieti perlu disertakan bersama Borang Soalselidik Teknikal ini.

A representative colour photograph of the variety should accompany the Technical Questionnaire.

Pihak berkuasa mungkin membenarkan maklumat tertentu ini diberi dalam bahagian sulit Borang Soal Selidik Teknikal.

Authority may allow certain of this information to be provided in a confidential section of the Technical Questionnaire.

8. KEBENARAN PENGELUARAN

AUTHORIZATION FOR RELEASE

8. (a) Adakah varieti memerlukan kebenaran sebelum pengeluaran di bawah undang-undang berhubung dengan perlindungan alam sekitar, kesihatan manusia dan kesihatan haiwan?

Does the variety require prior authorization for release under legislation concerning the protection of the environment, human and animal health?

☐ Ya *Yes* ☐ Tidak *No*

(b) Adakah kebenaran tersebut telah diperolehi?

Has such authorization been obtained?

☐ Ya *Yes* ☐ Tidak *No*

Jika jawapan kepada (b) ialah ya, sila kepilkan satu salinan kebenaran tersebut.

If the answer to (b) is yes, please attach a copy of the authorization.

9. MAKLUMAT BAHAN TANAMAN UNTUK DIPERIKSA ATAU DISERAH BAGI PEMERIKSAAN
INFORMATION ON PLANT MATERIAL TO BE EXAMINED OR SUBMITTED FOR EXAMINATION

9.1 Ekspresi satu ciri atau beberapa ciri varieti mungkin terjejas oleh faktor-faktor seperti perosak dan penyakit, rawatan kimia (contohnya bahan perencat pertumbuhan atau racun perosak), kesan pengkulturan tisu, akar stok berlainan, skion yang diambil daripada fasa pertumbuhan pokok yang berlainan dan lain-lain.

The expression of a characteristic or several characteristics of a variety may be affected by factors, such as pests and disease, chemical treatment (e.g. growth retardants or pesticides), effects of tissue culture, different rootstocks, scions taken from different growth phases of a tree, etc.

9.2 Bahan tanaman tidak seharusnya melalui apa-apa rawatan yang menjejaskan ekspresi ciri varieti, kecuali rawatan tersebut dibenarkan atau diminta oleh pihak berwibawa. Jika bahan tanaman telah melalui rawatan sedemikian, butir-butir penuh rawatan mestilah diberikan. Berhubung dengan hal ini, sila tunjukkan di bawah ini, sepanjang pengetahuan anda, sekiranya bahan tanaman untuk diperiksa itu tertakluk kepada:

The plant material should not have undergone any treatment which would affect the expression of the characteristics of the variety, unless the competent authority allows or requests such treatment. If the plant material has undergone such treatment, full details of the treatment must be given. In this respect, please indicate below, to the best of your knowledge, if the plant material to be examined has been subjected to:

(a) mikroorganisma (contohnya virus, bakteria, fitoplasma)
Microorganisms (e.g. virus, bacteria, phytoplasma)

☐ Ya
Yes ……

Sila berikan butir-butir bagi jawapan "ya" yang anda berikan.
Please provide details for where you have indicated "yes"

☐ Tidak
No

(b) rawatan kimia (contohnya bahan perencat pertumbuhan, racun perosak)
chemical treatment (e.g. growth retardant, pesticide)

☐ Ya
Yes ……

Sila berikan butir-butir bagi jawapan "ya" yang anda berikan.
Please provide details for where you have indicated "yes"

☐ Tidak
No

(c) kultur tisu
tissue culture

☐ Ya
Yes ……

Sila berikan butir-butir bagi jawapan "ya" yang anda berikan.
Please provide details for where you have indicated "yes"

☐ Tidak
No

(d) faktor-faktor lain
other factors

☐ Ya
Yes ……

Sila berikan butir-butir bagi jawapan "ya" yang anda berikan.
Please provide details for where you have indicated "yes"

☐ Tidak
No

10. PENGISYTIHARAN

DECLARATION

Saya dengan ini mengisytiharkan, sepanjang pengetahuan saya, bahawa maklumat yang diberi dalam borang ini adalah betul.

I hereby declare that, to the best of my knowledge, the information provided in this form is correct.

_____

Tandatangan

Signature

Nama pemohon : _____

Applicant's name

Tarikh : _____

Date

PENGHARGAAN
ACKNOWLEDGEMENT

Kelapa sawit adalah tanaman ladang yang penting kepada ekonomi negara dengan penghasilan minyak per unit kawasan yang tertinggi. Kelapa sawit telah melalui banyak penambahbaikan dan telah menghasilkan banyak varieti baka yang bermutu. Permintaan kelapa sawit yang semakin meningkat telah menarik perhatian sejumlah makmal bebas tanpa skim pembiakbakaan yang terancang untuk menghasilkan bahan tanaman kelapa sawit. Sehubungan itu, wujudnya terbitan bahan tanaman dari sumber yang tidak diperakui. Oleh itu, adalah penting untuk membangunkan satu garis panduan Ujian Kelainan, Keseragaman dan Kestabilan bagi kelapa sawit (DUS-OP). Akta Perlindungan Varieti Baru Tumbuhan 2004 menyediakan suatu landasan untuk melindungi hak milik pembiakbaka dan seterusnya mengalakkan pembiakbakaan varieti baru.

Oil palm is an economically important plantation crop with the highest oil yield per unit area. Over the years, the oil palm has gone through series of improvements and these have given rise to many quality breeding varieties. The growing demand for oil palm has attracted many independent laboratories without the support of a dedicated breeding programme to produce oil palm planting materials. As such, there is apprehension that the planting materials produced may have derived from non bona fide sources. Therefore, there is an urgent need to establish the distinctness, uniformity and stability test for oil palm (DUS-OP). The Protection of New Plant Varieties Act 2004 provides a platform for the protection of plant breeders' rights and thus promoting breeding of new varieties.

Satu jawatankuasa yang terdiri daripada pembiakbaka, ahli kultur tisu dan ahli biologi molekul dari pelbagai agensi dibentuk untuk menghasilkan garis panduan ujian DUS kelapa sawit. Senarai ahli jawatankuasa tersebut adalah seperti berikut:

In developing the guidelines for testing DUS for oil palm, a Committee was formed consisting of breeders, tissue culturists and molecular biologists from various agencies. Below is the list of the members of the Committee:

Jawatankuasa Utama
Main Committee

Dr. A. Kushairi Din (MPOB)
En. (Mr.) Cheah Lee Shen (DOA)
Dr. S. Ravigadevi (MPOB)
Dr. Soh Aik Chin (AAR)
En. (Mr.) Chin Cheuk Weng (FELDA)
Dr. N. Rajanaidu (MPOB)
Dr. Meilina Ong Abdullah (MPOB)
Dr. Sharifah Shahrul Rabiah Syed Alwee (FELDA)
En. (Mr.) Mustafa Kamal Mohamed (Sime Darby)
En. (Mr.) Mohd Isa Zainol Abidin (Kulim Bhd)
Dr. Lim Loon Lui (IOI)
Dr. Rajinder Singh (MPOB)
Cik (Ms.) Florence Ginibun (DOA)
Dr. Hamidah Musa (Sime Darby)
Pn. (Mrs.) Junaidah Judin (FELDA)
Dr. Mohd Din Amiruddin (MPOB)
Dr. Choong Chieh Wean (ASIATIC)
En. (Mr.) Wong Choo Kien (AAR)
En. (Mr.) Esa Sulaiman (DOA)
En. (Mr.) Kadir Zainal (DOA)

MPOB: Malaysian Palm Oil Board, AAR: Applied Agricultural Resources Sdn. Bhd., FELDA: Federal Land Development Authority, IOI: IOI Corporation Bhd., Sime Darby: Sime Darby Berhad, DOA: Department of Agriculture

Satu Jawatankuasa Kerja turut dibentuk untuk meneliti dan memastikan kriteria DUS ditepati dalam garis panduan kelapa sawit. Ahli Jawatankuasa Kerja adalah seperti berikut:

A Working Committee was also formed to look into the finer details of the requirements for DUS of oil palm. Below is the list of members of the Working Committee:

Jawatankuasa Kerja

Working Committee

Pn.(Mrs.) Junaidah Judin (FELDA)
Dr. Mohd Din Amiruddin (MPOB)
En.(Mr.) Mustafa Kamal Mohamed (Sime Darby)
En. (Mr.) Mohaimi Mohamed (Sime Darby)
En. (Mr.) Musa Bilal (UP)
En.(Mr.) Mohd Isa Zainol Abidin (Kulim Bhd)
En.(Mr.) Wong Choo Kien (AAR)
En.(Mr.) Marhalil Marjuni (MPOB)
Dr. Meilina Ong Abdullah (MPOB)
Cik (Ms.) Sudarti Asri (DOA)
Cik (Ms.) Soo Foong Lian (DOA)

Jawatankuasa Utama berfungsi mulai suku tahun pertama 2006. Jawatankuasa Kecil (Jawatankuasa Kerja) membangunkan kandungan garis panduan termasuk piawaian tatacara pelaksanaan. Garis panduan ini seterusnya diteliti oleh Jawatankuasa Utama Draf Garis Panduan bagi Pelaksanaan Ujian DUS Kelapa Sawit dan telah diluluskan oleh Lembaga Varieti Tumbuhan.

The Committees took effect in the first quarter of 2006. The Sub-Committee (Working Committee) developed detailed descriptions of the guidelines as well as the standard operating procedures. These guidelines were then fine-tuned by the Committee. The draft of the guidelines for the conduct of tests for DUS of oil palm was tabled and endorsed during the Plant Varieties Board Meeting.

ANNEX 1

TATACARA
ANALISIS TANDAN KELAPA SAWIT

PROCEDURE
OIL PALM BUNCH ANALYSIS

JABATAN PERTANIAN MALAYSIA
DEPARTMENT OF AGRICULTURE MALAYSIA

Februari 2010
February 2010

ANNEX 1 – Useful Explanations on OIL PALM BUNCH ANALYSIS

PART I
INTRODUCTION

The following Annex contains a description of the method to be used for OIL PALM bunch analysis. Usually, bunch analysis is done to determine the quality of oil palm bunches and fruit components for commercial purposes. Applicant of New Plant Variety Protection may submit the result of bunch analysis as an additional supporting document for dispute settlement.

However, this result of analysis has no decisive right in differentiating varieties due to the characteristics presented in bunch analysis may not fulfill the criteria set in UPOV document TGP/7/1: Development of Test Guidelines.

As for applicants who would like to enclose their application together with a set of bunch analysis of their applied varieties should followed the procedure recommended by Malaysia Palm Oil Board (MPOB) as stated below.

PART II
Description of the Method to be Used

OIL PALM BUNCH ANALYSIS

1.0 MATERIALS REQUIRED:

Sampling is done after 4 years of field planting. A minimum of 3 bunches are sampled from each individual palm.

2.0 BUNCH ANALYSIS STANDARD OPERATING PROCEDURES (SOP):

Day 0

1. Do cut test to determine fruit form and harvest a ripe bunch (minimum of 10 loose fruits after harvesting and minimum bunch weight of 5 kg from normal bunch) from selected palms.

2. Put harvested bunches into separate gunnysacks and bring to the laboratory. Bunch collection should commence at least 42 months after field planting.

3. Again, do another cut test to reconfirm the fruit form.

4. Record date, analysis serial number and fruit form, etc. (*Appendix 1*).

5. Weigh fruit bunch sample together with its loose fruits. In the case of any unavoidable delay (> five hours, e.g.: outstation trials), bunches should be weighed immediately in the field.

6. Chop the bunch and weigh the stalk. Stalk (peduncle) is cut to lowest spikelet before bunch is weighed in the laboratory.

7. Push spikelets into compartment boxes to separate them into various fractions, to get a representative random sample. Numbers of boxes involved in sampling of spikelets for fruit to bunch (F/B) and fruit components (FC) are done according to the bunch weight chart (*Table 1*).

8. Weigh and keep F/B sub-sample (spikelets) for two days in room temperature to ease fruit detachment.

9. Preparation for FC sub-sample (fertile fruits only):

   a). Separate fruits from spikelets using knife and remove calyxes.

   b). Pour fruit sub-samples evenly into dividing boxes. Take 30 – 40 fruits (about 300 g) at random depending on the fruit size. Count and weigh the fruit sub-samples.

   c). Use a ractangular box at the final stage of randomization if the fruits are in abundance. Arrange the fruits linearly along the perimeter of the box and start picking required fruit samples from one end.

   d). Do not take any cut and bruised fruits but replace with fruits of about equal dimensions and ripeness appearance.

Table 1: Bunch Weight Chart

Bunch Weight (kg)	No. of Compartment Boxes	
	* F/B	** FC
> 41	1/8	1/8
24 – 40	2/8	1/8
17 – 23	3/8	1/8
12 – 16	4/8	1/8
< 11	3/4	1/4
*F/B = Fruit to Bunch **FC = Fruit Components		

10. Scrape (depericarp) fruits into thin slices to separate nuts from mesocarp. Oil loss during fruit depericarping should be minimized.

11. Take and weigh all the fresh mesocarp. Keep in drying oven at 105°C for 16 hours.

12. Weigh fresh nut and dry in oven at 90°C (*dura*) and 80°C (*tenera*) for 16 hours.

Day 1

1. Remove dry mesocarp from oven, cool in a desiccator for about 30 minutes. Then weigh the dry mesocarp.

2. Mince mesocarp in a blender and sieve with 0.125" mesh.

3. Take 5 g sample for oil extraction (*Appendix 2b*).

4. Remove nuts from drying oven. Crack nuts and weigh the kernels.

Day 2 (F/B samples)

1. Separate fruits from spikelets manually (by hand).

2. Weigh fertile fruits, oil bearing parthenocarpic fruits and empty spikelets separately. Infertile fruits (colourless and non-oil bearing) are considered as part of empty spikelet components. *Appendix 5* illustrates the flow chart for bunch analysis

Appendix 1

A SAMPLE OF BUNCH ANALYSIS RAW SHEET

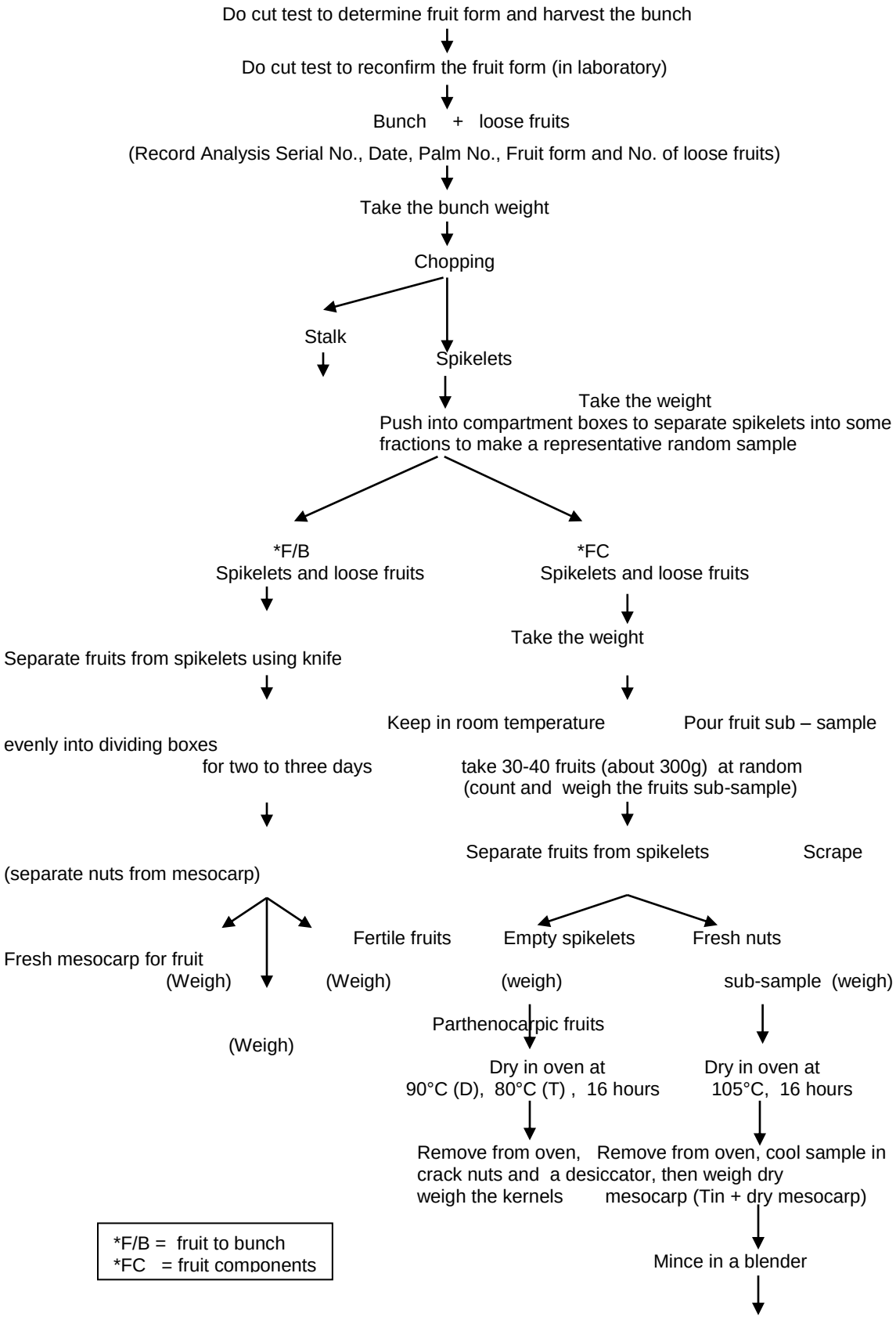
Lembaga Minyak Sawit Malaysia

MALAYSIAN PALM OIL BOARD

BUNCH ANALYSIS

Analysis Serial No.		6	4	7	1	0	
Date	1	0	0	7	0	5	
Expt. No.				3	9	0	
Palm No.			1	0	5	9	
Fruit Type						T	
No. of Loose Fruit at harvest					1	0	
Bunch Weight			8	7	1	0	kg
Stalk Weight			1	0	1	0	kg
Spikelet Weight			4	0	8	0	kg
Fertile Fruit Weight			2	7	4	0	kg
Parthenocarpic Fruit Wt.	2	3	0	0			g
Empty Spikelet Wt. **1**	0	0	0	0			g
Fruit Sub Sample Wt.	3	0	0	8			g
Fresh Nut Weight		4	7	7			g
No. of Fresh Nuts		2	1				
Kernel Weight		2	6	4			g
Tin No.						1	
Tin Weight		6	2	0			g
Tin+Dry Mesocarp Wt.	2	1	7	9			g
Extraction Thimble Wt.			1	4	8	0	g
Extraction Thimble + Mesocarp			6	4	7	0	g
Extraction Thimble + Fibre Wt.			2	5	5	0	g
Wet Mesocarp Wt.							g

FLOW CHART OF BUNCH ANALYSIS METHOD (*DURA* AND *TENERA*)



** Take 5 g sampel for oil extraction

(continued in *Appendix 2b*)

Appendix 2b

**** Oil extraction of 5 g mesocarp samples**

Mince mesocarp

↓

Prepare extraction thimble[1] measuring 7.5 x 15 cm

↓

Weigh 5 g mesocarp (analytical balance – readability : 4 decimal places)

↓

Put mesocarp sample into extraction thimble[1], pack and staple

↓

Put mesocarp sample (in extraction thimble) into oven (40° C) for two hours

↓

Weigh sample (mesocarp & extraction thimble)

↓

Extract oil using soxhlet extractor[2] with solvent (haxene)

↓

Extract for 18 – 24 hours (until the solvent turns into its original colour)

↓

Remove sample from soxhlet extractor

↓

Dry it in oven (105°C) for 16 hours

↓

Weigh fibre & extraction thimble

---

[1]*Filter paper Whatman No. 1*

[2]*Soxhlet extraction set:*

- *Eletrothermal heating mantle (5 l capacity)*
- *Round bottom flask: (5 l capacity)*
- *Extractor: (2 l capacity)*
- *Condenser*

Appendix 3

COMPUTATION FORMULAE FOR BUNCH ANALYSIS COMPONENTS (*DURA* & *TENERA*)

F/B	= Fruit/Bunch (%)	= [FFWT + PFWT)/SWT] x [(BWT - STKWT)/BWT] x 100
FF/B	= Fertile Fruit/Bunch (%)	= [(FFWT/SWT) x ((BWT - STKWT)/BWT)] x 100
P/F	= Parthenocarpic/Fruit (%)	= [PFWT/(FFWT + PFWT)] x 100
M/F	= Mesocarp/Fruit (%)	= [(FSWT - FNWT)/FSWT] x 100
MC	= Moisture Content (%)	= 100 - [(TDMWT - TWT)/(FSWT – FNWT)] x 100
O/DM	= Oil/Dry Mesocarp (%)	= [(ETMWT - ETFWT)/(ETMWT - ETWT)] x 100
O/WM	= Oil/Wet Mesocarp (%)	= [((TDMWT–TWT)/(FSWT– FNWT)) x O/DM]/100
O/B	= Oil/Bunch (%)	= (F/B x M/F x O/WM)/10,000
O/F	= Oil/Fibre (%)	= [(ETMWT - ETFWT)/(ETFWT – ETWT)] x 100
K/F	= Kernel/Fruit (%)	= (KWT/FSWT) x 100
S/F	= Shell/fruit (%)	= [(FNWT – KWT)/FSWT] x 100
K/B	= Kernel/Bunch (%)	= (K/F x FF/B) /100
MNW	= Mean Nut Weight (g)	= FNWT/NOFNUT
MFW	= Mean Fruit Weight (g)	= FSWT/NOFNUT
P/B	= Parthernocarpic/Bunch (%)	= (P/F x F/B)/100
OY	= Oil yield (kg/p/yr)	= (FFB x O/B)/100
KY	= Kernel yield (kg/p/yr)	= (FFB x K/B)/100
TOT	= Total Oil (kg/p/yr)	= OY + (0.5 x KY)
TEP	= Total economic product (kg/p/yr)	= OY + (0.6 x KY)

Where:

BWT	= Bunch Weight	STKWT	= Stalk Weight
SWT	= Spikelet Weight	FFWT	= Fertile Fruit Weight
PFWT	= Parthenocarpic Fruit Weight	ESPKWT	= * Empty Spikelet Weight
FSWT	= Fruit Sub Sample Weight	FNWT	= Fresh Nut Weight
NOFNUT	= No of Fresh Nut	KWT	= Kernel Weight
TDMWT	= Tin + Dry Mesocarp Weight	TWT	= Tin Weight
ETWT	= Extraction Thimble Weight	FFB	= Fresh Fruit Bunch
ETMWT Weight	= Extraction Thimble + Mesocarp Weight	ETFWT	= Extraction Thimble + Fibre

* ESPKWT = Empty Spikelet + infertile fruit (colourless and non-oil bearing)

Appendix 3a

SIMPLIFIED FORMULAE FOR BUNCH ANALYSIS COMPONENTS (*DURA* & *TENERA*)

F/B	= Fruit/Bunch (%)	= [FFWT + PFWT)/SWT] x [(BWT - STKWT)/BWT] x 100
FF/B	= Fertile Fruit/Bunch (%)	= [(FFWT/SWT) x ((BWT - STKWT)/BWT)] x 100
P/F	= Parthenocarpic/Fruit (%)	= [PFWT/(FFWT + PFWT)] x 100
M/F	= Mesocarp/Fruit (%)	= [(FSWT - FNWT)/FSWT] x 100
MC	= Moisture Content (%)	= 100 - [(DMWT)/(WMWT)] x 100
O/DM	= Oil/Dry Mesocarp (%)	= [(MWT - FWT)/(MWT] x 100
O/WM	= Oil/Wet Mesocarp (%)	= [(DMWT)/(WMWT) x O/DM]/100
O/B	= Oil/Bunch (%)	= (F/B x M/F x O/WM)/10,000
O/F	= Oil/Fibre (%)	= [(MWT - FWT)/(FWT)] x 100
K/F	= Kernel/Fruit (%)	= (KWT/FSWT) x 100
S/F	= Shell/fruit (%)	= [(FNWT – KWT)/FSWT] x 100
K/B	= Kernel/Bunch (%)	= (K/F x FF/B) /100
MNW	= Mean Nut Weight (g)	= FNWT/NOFNUT
MFW	= Mean Fruit Weight (g)	= FSWT/NOFNUT
P/B	= Parthernocarpic/Bunch (%)	= (P/F x F/B)/100
OY	= Oil yield (kg/p/yr)	= (FFB x O/B)/100
KY	= Kernel yield (kg/p/yr)	= (FFB x K/B)/100
TOT	= Total Oil (kg/p/yr)	= OY + (0.5 x KY)
TEP	= Total economic product (kg/p/yr)	= OY + (0.6 x KY)

Where:

BWT	= Bunch Weight	STKWT	= Stalk Weight
SWT	= Spikelet Weight	FFWT	= Fertile Fruit Weight
PFWT	= Parthenocarpic Fruit Weight	ESPKWT	= * Empty Spikelet Weight
FSWT	= Fruit Sub Sample Weight	FNWT	= Fresh Nut Weight
NOFNUT	= No of Fresh Nut	KWT	= Kernel Weight
TDMWT	= Tin + Dry Mesocarp Weight	TWT	= Tin Weight
ETWT	= Extraction Thimble Weight	FFB	= Fresh Fruit Bunch
ETMWT	= Extraction Thimble + Mesocarp Weight	ETFWT	= Extraction Thimble + Fibre Weight

* ESPKWT = Empty Spikelet + infertile fruit (colourless and non-oil bearing)

Appendix 4

A sample of bunch analysis output

Table X: FAMILY MEAN FOR BUNCH QUALITY COMPONENTS OF TENERAS IN TRIAL Y - YEAR 1995 - 2003

DATE PLANTED: Jun. 91 MATERIAL: D x P BREEDING DESIGN: Bips STATISTICAL DESIGN: RCBD

SNO	PROG	PEDIGREE	N	BWT	MFW	MNW	P/B	M/F	K/F	S/F	O/DM	O/WM	F/B	O/B	K/B	MC	O/F	OY	KY	TOT	TEP
1	DP 9	x	16	12.89	11.63	2.39	5.54	79.26	10.33	10.40	79.95	51.49	69.78	28.51	7.21	35.66	404.15	35.85	9.16	40.44	41.35
2	DP 10	x	18	13.95	11.36	2.15	4.56	80.67	10.04	9.29	79.09	49.39	69.20	27.57	6.94	37.65	385.58	41.43	10.46	46.67	47.71
3	DP 12	x	18	12.33	13.29	2.78	8.13	78.41	10.52	11.07	80.36	49.38	67.26	25.97	7.11	38.63	413.98	32.61	9.17	37.19	38.11
4	DP 20	x	19	14.72	11.97	2.57	5.37	78.06	10.60	11.34	79.95	50.01	69.54	27.18	7.34	37.56	405.66	41.65	11.38	47.35	48.48
5	DP 23	x	19	13.92	11.57	2.38	4.67	78.91	9.83	11.26	80.96	51.31	70.94	28.75	6.98	36.69	430.52	39.28	9.43	43.99	44.93
6	DP 70	x	15	21.49	10.21	2.05	3.88	79.25	10.14	10.62	80.61	50.74	68.46	27.51	6.94	37.12	421.82	47.67	12.11	53.73	54.94
7	DP 34	x	18	15.33	14.41	2.62	5.52	81.31	9.23	9.46	79.74	49.10	70.67	28.19	6.51	38.49	399.06	44.11	10.10	49.16	50.17
8	DP 42	x	20	14.96	10.77	2.04	5.05	80.76	8.73	10.51	80.75	53.29	68.41	29.46	5.96	34.07	426.42	49.72	9.99	54.72	55.72
9	DP 44	x	20	15.17	11.82	2.19	4.79	81.25	9.04	9.71	80.66	50.43	68.32	28.07	6.17	37.56	426.50	48.21	10.67	53.55	54.61
10	DP 54	x	17	13.92	11.28	1.98	5.55	81.76	7.91	10.33	80.46	51.94	67.22	28.60	5.32	35.52	419.04	50.24	9.44	54.97	55.91
11	DP 61	x	19	18.49	11.61	2.94	4.22	74.12	12.68	13.20	80.40	49.39	70.11	25.69	8.88	38.61	419.43	41.05	14.65	48.38	49.84
12	DP 67	x	18	19.33	10.75	2.30	4.22	78.39	10.53	11.08	79.33	47.47	68.36	25.49	7.16	40.27	392.26	43.91	12.62	50.23	51.48
13	DP 69	x	15	18.46	10.74	2.76	3.58	73.89	11.51	14.60	79.74	48.07	68.79	24.40	7.94	39.84	401.01	37.18	12.07	43.22	44.43
14	DP 72	x	19	13.99	10.50	1.96	5.41	80.85	8.85	10.30	80.62	49.73	68.45	27.60	6.05	38.39	425.61	45.81	10.10	50.86	51.87
15	DP 75	x	18	15.09	11.10	2.63	5.21	76.05	11.49	12.47	79.34	48.99	68.13	25.34	7.87	38.32	388.67	38.53	12.06	44.57	45.77
MEAN			322	15.51	11.40	2.35	4.94	78.92	10.02	11.06	80.02	50.03	68.68	27.13	6.88	37.57	407.70	42.31	10.77	47.70	48.77
SOURCE OF VARIATION			df	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS
BETWEEN POPULATIONS			17	97.21**	21.98**	1.72**	19.76*	100.54**	28.02**	30.03**	7.47**	43.63**	31.93**	36.92**	15.04**	53.55**	5007.36**	412.88**	48.50**	431.18**	437.83**
WITHIN POPULATIONS			304	26.27	3.99	0.27	10.74	13.91	3.46	5.55	2.92	11.83	10.84	7.28	1.75	11.04	1938.63	113.70	12.32	140.20	146.24
CV																					
LSD																					

LITERATURE

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ANNEX 2

TATACARA
PENGECAPJARIAN BAGI KELAPA SAWIT: Penerangan Penting Ciri-Ciri Molekular dengan Penggunaan Analisis Simple Sequence Repeat (SSR)

PROTOCOLS
DNA PROFILING OF OIL PALM :
Useful Explanations on Molecular Characteristics using Simple Sequence Repeat (SSR) Analysis

JABATAN PERTANIAN MALAYSIA
DEPARTMENT OF AGRICULTURE MALAYSIA

Februari 2010
February 2010

ANNEX 2 – Useful Explanations on Molecular Characteristics using Simple Sequence Repeat (SSR) Analysis

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PART I
INTRODUCTION

The following Annex contains a description of the method to be used, for analysis of samples using electrophoresis. The method described is to be used as a complement to other differences in morphological or physiological characteristics.

Procedure described hereafter can be carried out using simple sequence repeat (SSR) primers as reported for oil palm by MPOB (Singh *et al*., 2007)

A total of 10 SSR primers can be used for the analysis of the samples. At its maximum, the analysis of all 10 primers can be carried out simultaneously in the same day by using two electrophoresis sets (radioactive method) and one electrophoresis gel run (Licor Analysis) separately. For each SSR primer, there are a number of different possible alleles which appear as a series of well defined bands or patterns of bands. The analysis of the SSR primer is based on the recognition of these alleles as indicated in Table and Figures 1, 2, 3 & 4.

PART II
Description of the Method to be Used

OIL PALM GENOMIC DNA EXTRACTION METHODS

The two methods described here are recommended for routine use. Alternative methods that give good quality DNA may also be accepted.

A) OIL PALM GENOMIC DNA EXTRACTION METHOD FOR YOUNG/SPEAR LEAVES (MODIFIED CTAB BUFFER METHOD)

1.0 Apparatus and equipment

1.1 Refrigerated Floor Standing High Speed Centrifuge (Max Speed at least 20,000 rpm)

1.2 -20^{o}C freezer and -80 ^{o}C freezer

1.3 Refrigerator

1.4 Waterbath

1.5 Top-pan balance

1.6 Speedvac concentrator

1.7 Micropipettes (all range)

1.8 Sterile micropipette tips (with tip cut off)

1.9 Sterile corex tubes (15 ml, 30 ml)

1.10 Sterile mortar and pestle (13.5 cm or 16 cm diameter)

1.11 Sterile polypropylene tubes (50 ml, 250 ml)

1.12 Sterile graduated pipettes (10 ml, 25 ml)

2.0 Chemicals

All chemicals should be of 'Analytical Reagent' grade or better.

2.1 Chemicals for DNA extraction

2X CTAB buffer
0.5 M Ascorbic acid
0.4 M DIECA
PVP-40
2-mercaptoethanol
Chloroform/isoamyl alcohol (24:1)
Isopropanol
Wash buffer
TE buffer pH8.0
7.5 M Ammonium acetate pH7.7
Absolute ethanol
70 % ethanol
10 mg/ml RNase A
Acid-washed sand
Liquid nitrogen

3.0 Solutions

3.1 Solutions for DNA extraction

3.1.1 100 ml 2X CTAB buffer

10 ml	1 M Tris-HCl pH8.0
4 ml	0.5 M EDTA pH8.0
8.2 g	NaCl
2 g	Cetylmethyl-ammonium bromide (CTAB)

To dissolve at 65 ^{o}C. Sterile ultra-pure water (up to 100 ml when CTAB has dissolved). Autoclave before storage.

3.1.2 10 ml 7.5 M Ammonium acetate pH7.7

5.78 g Ammonium acetate (MW = 77.08)
Sterile Ultra-pure water (up to 10 ml)

Adjust pH with Ammonium hydroxide or Acetic acid
Sterilize by filtering through a sterile 0.22 micron cellulose nitrate membrane filter into an acid-washed bottle.
Store at 4 ^{o}C.

3.1.3 100 ml Wash buffer

76 ml Absolute ethanol
0.13 ml 7.5 M Ammonium acetate pH7.7

Sterile Ultra-pure water (up to 100 ml)
Store at -20 ^{o}C.

3.1.4 100 ml 0.5 M Ascorbic acid

8.805 g Ascorbic acid (MW = 176.1)
Sterile Ultra-pure water (up to 100 ml)

Filter sterilize through a sterile 0.22 micron cellulose nitrate membrane filter.
Store at 4 ^{o}C.

3.1.5 100 ml 0.4 M DIECA

6.852 g Diethyldithiocarbamic acid (DIECA) (FW = 171.3)
Sterile Ultra-pure water (up to 100 ml)

Filter sterilize through a sterile 0.22 micron cellulose nitrate membrane filter.
Store at 4 ^{o}C.

3.1.6 Extraction buffer

100 ml	2X CTAB buffer
2 g	PVP-40
1 ml	0.5 M Ascorbic acid
1 ml	0.4 M DIECA

3.1.7 1 L 10X TAE buffer
48.46 g Trizma base

3.72 g	EDTA, disodium
6.80 g	Sodium acetate trihydrate

3.1.8 400 ml TE buffer pH8.0

4 ml 1 M Tris-HCl pH8.0
80 ul 0.5 M EDTA pH8.0
Sterile Ultra-pure water (up to 400 ml)

3.1.9 10 mg/ml RNase
50 ul 1 M Tris-HCl pH7.5
15 ul 5 M NaCl
50 mg RNase A
4.93 ml Sterile Ultra-pure water

Dissolve RNase A. Heat to 100 ^{o}C for 15 min. Leave the tube in the boiled water to cool slowly to room temperature. Dispense into aliquots. Store at -20 ^{o}C.

4.0 Procedure

4.1 DNA Extraction (Modified CTAB buffer method)

(a) Prepare Extraction buffer and keep at 60 ^{o}C (to be prepared fresh when required).

(b) Grind 5 g leaves with sand (0.2 g) in liquid nitrogen.

(c) Let liquid nitrogen evaporate. Add 25 ml Extraction buffer and 0.5 ml of 2-mercaptoethanol. Swirl gently to mix.

(d) Transfer ground tissue to a 250 ml polypropylene bottle. Incubate extract in 60 ^{o}C waterbath for 30 min (swirl occasionally).

(e) Cool tube to room temperature. Add 25 ml chloroform/isoamyl alcohol (24:1). Mix gently by inverting tube.

(f) Centrifuge at 10,000 rpm at 4 ^{o}C for 15 min in a high speed centrifuge.

(g) Remove aqueous phase into a 50 ml polypropylene tube. Use a sterile graduated pipette or a blue tip with tip cut off. Note volume of aqueous phase.

(h) Add 0.6 volume of isopropanol. Mix content of tube. Allow nucleic acids to precipitate at -20 ^{o}C for at least 1 hr (or left overnight).

(i) Centrifuge to pellet at 12,000 rpm at 4 ^{o}C for 15 min. Decant supernatant. Drain pellet.

(j) Wash pellet by resuspending in 5-10 ml Wash buffer. Leave the tube for at least 1 hr until the pellet turns white.

(k) Spin to pellet. Decant supernatant. Drain pellet.

(l) Wash pellet again and repeat step (k). Dry pellet in Speedvac.

(m) Transfer the dried pellet into a 15 ml acid-washed, baked corex tube.

(n) Re-dissolve pellet in 4 ml TE buffer pH8.0 at 4 ^{o}C in refrigerator overnight or in 50 ^{o}C waterbath for 4 - 5 hrs.

(o) Add 5 ul RNase solution and incubate at room temperature for 30 min.

(p) Add 2 ml 7.5 M Ammonium acetate pH7.7. Mix content and leave the tube on ice for 20 min.

(q) Centrifuge at 12,000 rpm at 4 ^{o}C for 15 min. Transfer the RNase-treated sample to another 15 ml corex tube. Use a sterile graduated pipette or a blue tip with tip cut off. Note the volume of the RNase-treated sample.

(r) Add 2.5 volume of -20 ^{o}C ethanol. Mix by inverting tube (cover with cling film) and keep tube overnight at -20 ^{o}C.

(s) Spin precipitate at 12,000 rpm at 4 ^{o}C for 15 min to pellet DNA.

(t) Wash pellet with 70 % ethanol. Spin. Drain pellet. Dry in Speedvac.

(u) Re-dissolve pellet in 2 - 3 ml TE buffer pH8.0.

(v) Estimate DNA concentration

(w) Check quality of DNA on a 0.9 % agarose gel in 1X TAE buffer.

B) <u>OIL PALM GENOMIC DNA EXTRACTION METHOD FOR MATURE OIL PALM LEAVES</u> (Seng & Faridah, 2006).

1.0 Apparatus and equipment

1.1 Refrigerated bench top centrifuge

1.2 -20^{o}C and -80 ^{o}C freezer

1.3 Waterbath (temperature adjustable from ambient to 100^{0}C)

1.4 Top-pan balance

1.5 Micropipettes (all range)

1.6 Sterile micropipette tips (all range)

1.7 Sterile polypropylene tubes (15 ml, 50 ml)

1.8 Sterile eppendorf tubes (1.5ml)

1.9 Mortar and pestle (13.5 cm or 16 cm diameter)

2.0 Chemicals

All chemicals should be of 'Analytical Reagent' grade or better.

2.1 Chemicals for DNA extraction

2X CTAB buffer
Nucleic extraction buffer (NEB)
Nucleic lysis buffer (NLB)
1M Tris-HCl (pH8)
0.5M EDTA
Polyvinylpyrolidone (PVP-40)
N-Lauroylsarcosine
4M D-Sorbitol
0.5 M Ascorbic acid
0.4 M DIECA
0.02M Sodium bisulphate
2- mercaptoethanol
Chloroform: Isoamyl alchohol (24:1)
Isopropanol

TE buffer pH8.0
Wash buffer
7.5M ammonium acetate, pH7.7
Absolute Ethanol
Liquid nitrogen
10 mg/ml RNase A
70 % ethanol

3.0 Solutions

3.1 Solutions for preparation of DNA extraction Method B

3.1.1 1L 2X CTAB buffer

100ml 1M Tris-HCl, pH8.0
40ml 0.5M Na_2EDTA, pH8.0
82g NaCl
2g cetylmethyl-ammonium bromide (CTAB)

Autoclave at 121°C for 20min
Store at room temperature

3.1.2 500ml 1M Tris-HCl (pH8)

60.57g Tris-HCl
Sterile Ultra-pure water (up to 500ml)

Adjust pH with ammonium hydroxide or acetic acid
Autoclave at 121°C for 20min
Store at room temperature

3.1.3 1L 0.5M Na_2EDTA (pH8)

185.12g Na_2EDTA
Sterile Ultra-pure water (up to 1L)

Adjust pH with ammonium hydroxide or acetic acid
Autoclave at 121°C for 20min
Store at room temperature

3.1.4 250ml 4M D- Sorbitol

182.17g D- Sorbitol
Sterile Ultra-pure water (up to 500ml)

Filter sterilize through a sterile 0.22 micron cellulose nitrate membrane filter
Store at room temperature

3.1.5 10ml 7.5M Ammonium Acetate, pH7.7

5.78g Ammonium Acetate (MW = 77.08)
Sterile Ultra-pure water (up to 10ml)

Adjust pH with ammonium hydroxide or acetic acid
Filter sterilize solution with a sterile 0.22 micron cellulose nitrate membrane into an acid-washed bottle.
Store at 4^oC

3.1.6 100ml Wash buffer

76ml Absolute ethanol
0.13ml 7.5M ammonium acetate

Sterile Ultra-pure water (up to 100ml)

Store at -20°C.

3.1.7 100ml 0.5M Ascorbic acid

8.805g Ascorbic acid
Sterile Ultra-pure water (up to 100ml)

Filter sterilize through a sterile 0.22 micron cellulose nitrate membrane filter
Store at 4°C

3.1.8 100ml 0.4M DIECA

6.852g Diethyldithiocarbamic acid (DIECA)
Sterile Ultra-pure water (up to 100ml)

Filter sterilize through a sterile 0.22 micron cellulose nitrate membrane filter
Store at 4°C

3.1.9 400 ml TE buffer pH8.0

4ml 1M Tris-HCl pH8.0
80ul 0.5M EDTA
Sterile Ultra-pure water (up to 400ml)

3.1.10 10 mg/ml RNase
50ul 1M Tris-HCl pH7.5
15ul 5M NaCl
50mg RNase A
4.93ml Sterile Ultra-pure water

Dissolve RNase A in sterile Ultra-pure water.
Heat to 100°C for 15 min and leave the tube in the boiled water to cool slowly to room temperature. Dispense into aliquots.
Store at -20°C.

3.1.11 120ml Nucleic Extraction Buffer
12 ml 1M Tris- HCl
4.8 ml 0.5M EDTA
2.4 g PVP-40
10.5 ml 4M D-Sorbitol
1.2 ml 0.4M DIECA
1.2 ml 0.5M Ascorbic acid
2.4 ml 1M Sodium bisulphate
Sterile Ultra-pure water (up to 120ml)

Autoclave at 121°C for 20min
Store at room temperature

3.1.12 20 ml Nucleic Lysis Buffer
120 ml 2X CTAB Buffer
2.4 g PVP-40
1.2 g N-Lauroylsarcosine
4.2 ml 4M D-Sorbitol
1.2 ml 0.4M DIECA
1.2 ml 0.5M Ascorbic acid
1.2 ml 2- mercaptoethanol

4.0 Procedure

4.1 DNA Extraction Method B

(a) Grind approximately 5 g fresh leaf in liquid nitrogen using mortar and pestle.

(b) Transfer the fine powder into 50 ml sterile polypropylene tube with 5ml ice-cold Nucleic Extraction buffer.

(c) Swirl gently and keep on ice for 15 min.

(d) Centrifuge the tubes at 8,000 rpm for 10 min at 4 ^{0}C.

(e) Discard the supernatant and save the pellet.

(f) Add ten mililiters Nucleic Lysis Buffer to resuspend the pellet.

(g) Incubate the tubes at 65 ^{0}C in the water bath for 30 min.

(h) Leave the tube to stand at room temperature for about 15 min.

(i) Add equal volume of chloroform:isoamylalcohol mixture (24:1) and invert the tube several times and centrifuge at 8,000 rpm for 15 min at room temperature.

(j) Transfer the supernatant into a new tube.

(k) Repeat the chloroform:isoamylalcohol extraction step twice. Precipitate the extracted DNA by adding equal amounts of pre-chilled isopropanol to the supernatant followed by incubation at –80 ^{0}C for 30 min.

(l) Pellet the aggregate nucleic acid by centrifugation at 4 ^{0}C, 10,000 rpm for 15 min.

(m) Discard the supernatant and save the pellet.

(n) Add 5 ml Wash buffer into the tube.

(o) After centrifugation, decant the Wash buffer and dry the pellet in the laminar airflow.

(p) Resuspend the pellet in 2 ml of Low TE buffer, pH8.0.

(q) Add RNase (10 mg/ml) into the DNA solution to a final concentration of 20 µg/ml and incubate at 37 ^{0}C for 30 min.

(r) Add half–volume of ammonium acetate, 7.5 M and mix evenly.

(s) Incubate the mixture on ice for 30 min and then centrifuge for 15 min at 10,000 rpm at 4 ^{0}C.

(t) Remove the clear upper phase supernatant without interfering with the pellet at the bottom and transfer to a new tube.

(u) Add 2.5 volume of absolute ethanol (-20 ^{0}C) and mix well by inverting the tube. Incubate the tube at –80 ^{0}C for 45 min then centrifuge for 10 min at 10,000 rpm at 4 ^{0}C.

(v) Discard the supernatant and wash the pellet using 5 ml 70% ethanol. Centrifuge the mixture for 10 min at 10,000 rpm at 4 ^{0}C.

(w) Dry the pellet thoroughly and redissolve in 1 ml of TE buffer and transfer into a sterile 1.5 ml Eppendorf tube.

(x) Quantity DNA with NanoDrop NP-1000 to determine the purity and quantity of the total extracted DNA.

(y) Analyse the integrity of total DNA through 0.8% agarose gel electrophoresis in 1 X TBE buffer.

(z) Keep the DNA at 4 ºC until needed.

5.0 Primers

5.1 Primer for Simple Sequence Repeat (SSR) Analysis (Radioactive Method)

Table 1: SSR loci, primer sequences and optimum annealing temperatures

Locus name	Primer sequences F: 5'-3' R: 5'-3'	Expected size of polymorphic allele (bp)	Annealing temperature (ºC)
sEg00097 (EAP 03745)	Fwd (left) : GAAGAAGGGTGTAGATGGTTC Rev (right) : CCTGAGCTTCATTGTCTGAT	176	52
sEg00056 (CNI01714)	Fwd (left) : ATACAATATGCTGCCTGAAG Rev (right) : TGCAATTTCTTAGCAGAAGC	170	52
sEg00038 (CNH01303)	Fwd (left) : ATCAAGCGGCAGTTATGAGAT Rev (right) : ATACATTATTCCCACCACCA	165	52
sEg00098 (EAP03854)	Fwd (left) : TTACAGTCGGGAACTATTGG Rev (right) : CATAAGAAGCAACCTCTGAA	174	52
sEg00167 (CNHP00167)	Fwd (left) : ACAAAAGATGAAAGCTGAAAAG Rev (right) : TACCAAACAAGAAAGCAGTTTA	155	52
sEg00032 (CEOP00030)	Fwd (left) : CTGTTGAGCTGGAGAGACCC Rev (right) : CCAACCAGGATCAGTTTGGT	269	55
sEg00080 (EAP01840)	Fwd (left) : AAGAACTATGACCTCACCAAAA Rev (right) : AACTCTATGCTATTGCTACACGA	153	52
sEg00125 (EO02861)	Fwd (left) : TACCCTTTTCCCTCCCTCCATA Rev (right) : CATCATCTCCGTTGCCAGTATT	152	52
sEg00126	Fwd (left) :		

(EO02978)	CCGTCTCAAAAGCCCTAAAC Rev (right) : TTGTTGTCCCACTCCCTCTT	216	52
sEg00127 (EO03035)	Fwd (left) : CTAAAATTCCCTCATCGTCTC Rev (right) : CTCGAAGCTCATCGTCTCTC	157	52

5.2 Primers for Simple Sequence Repeat (SSR) Analysis (LI-COR 4200 LONG READ NEN® GLOBAL EDITION IR2 SEQUENCER Method)

M13–tailed primer sequences, primer sequences, loci and optimum annealing temperatures

Locus name	**M13- tailed primer sequences** **F: 5'-3'** **R: 5'-3'**	**Expected size of polymorphic allele attached with M13 tail (bp)**	**Annealing temperature (°C)**
sEg00097 (EAP 03745)	Fwd (left): GGAAACAGCTATGACCATGAAGAAGGGTGTAGATGGTTC Rev(right): CCTGAGCTTCATTGTCTGAT	194	52
sEg00056 (CNI01714)	Fwd (left): GGAAACAGCTATGACCATATACAATATGCTGCCTGAAG Rev (right): TGCAATTTCTTAGCAGAAGC	188	52
sEg00038 (CNH01303)	Fwd (left) : GGAAACAGCTATGACCATATCAAGCGGCAGTTATGAGAT Rev (right): ATACATTATTCCCACCACCA	183	52
sEg00098 (EAP03854)	Fwd (left) : GGAAACAGCTATGACCATTTACAGTCGGGAACTATTGG Rev (right): CATAAGAAGCAACCTCTGAA	192	52
sEg00167 (CNHP00167)	Fwd (left) : GGAAACAGCTATGACCATACAAAAGATGAAAGCTGAAAAG Rev (right): TACCAAACAAGAAAGCAGTTTA	173	52
sEg00032 (CEOP00030)	Fwd (left) : GGAAACAGCTATGACCATCTGTTGAGCTGGAGAGACCC Rev (right) : CCAACCAGGATCAGTTTGGT	287	55
sEg00080 (EAP01840)	Fwd (left) : GGAAACAGCTATGACCATAAGAACTATGACCTCACCAAAA Rev (right) : AACTCTATGCTATTGCTACACGA	171	52
sEg00125 (EO02861)	Fwd (left) : GGAAACAGCTATGACCATTACCCTTTTCCCTCCCTCCATA Rev (right) : CATCATCTCCGTTGCCAGTATT	170	52
sEg00126 (EO02978)	Fwd (left) : GGAAACAGCTATGACCATCCGTCTCAAAAGCCCTAAAC Rev (right) : TTGTTGTCCCACTCCCTCTT	234	52
sEg00127 (EO03035)	Fwd (left) : GGAAACAGCTATGACCATCTAAAATTCCCTCATCGTCTC Rev (right) : CTCGAAGCTCATCGTCTCTC	175	52

SIMPLE SEQUENCE REPEAT (SSR) ANALYSIS

Two methods are described for SSR analysis. Alternative methods can be employed but are subjected to approval by the Plant Varieties Board.

METHOD 1

SSR ANALYSIS (RADIOACTIVE METHOD)

1.0 Number of samples per test
Maximum 45 samples per gel

2.0 Apparatus and equipment

2.1 Waterbath (temperature adjustable from ambient to 100^0C)

2.2 Top-pan balance

2.3 PCR machine

2.4 Vertical Gel Electrophoresis Apparatus (Life Technologies, Model S2 or equivalent)

Components:
- 2.4.1 Electrophoresis apparatus with upper buffer chamber, buffer chamber drain valve, silicone gasket, removable lower buffer tray, integral gel clamps, and safety lids
- 2.4.2 One pair of 122 cm (48 in.) DC power cords
- 2.4.3 One pair of glass plates (short plate: 32.5 cm x 39.5 cm,long plate: 32.5 cm x 42 cm)
- 2.4.4 One pair of 0.4 mm thick vinyl side spacers
- 2.4.5 One piece of 0.4 mm thick vinyl sharkstooth combs (48 wells)
- 2.4.6 One pair of adhesive-backed foam blocks for side spacers

2.10 Micropipettes (all range)

2.11 Refrigerated bench top centrifuge

2.12 Gel Dryer with vacuum system (40 x 50 cm Gels; 120VAC / 60Hz; Max temperature 80 ^{o}C)

2.13 X-ray film (Kodak)

3.0 Chemicals

All chemicals should be of 'Analytical Reagent' grade or better.

3.1 Chemicals for labeling of primers

10X T4 Kinase Reaction buffer (NEB)
T4 Polynucleotide Kinase (NEB)
^{33}PdATP

3.2 Chemicals for preparation of DNA Marker

5X Forward Exchange buffer (Invitrogen)
T4 Polynucleotide Kinase (NEB)
^{33}PdATP
TE buffer pH 8.0
Formamide
Bromophenol blue
Xylene cyanol
0.5 M EDTA pH 8.0

3.3 Chemicals for Polymerase Chain Reaction (PCR)

10X Standard *Taq* buffer (NEB)
10mM dNTP mix
Taq DNA Polymerase (NEB)

3.4 Chemicals for electrophoresis

Absolute ethanol
Acrylamide
Bis-acrylamide
Urea
Trizma base
Boric acid
0.5 M EDTA pH8.0
TEMED
Ammonium Persulphate
1X TBE buffer
Formamide dye

3.5 Chemicals for developing X-Ray film

KODAK GBX Developer
KODAK GBX Fixer

4.0 Solutions

4.1 Solutions for labeling of primers

4.1.1 10X T4 Kinase Reaction buffer (NEB)

700 mM Tris-HCl
100 mM $MgCl_2$
50 mM Dithiothreitol
pH 7.6 @ 25°C

4.2 Solutions for preparation of DNA marker

4.2.1 5X Forward Exchange buffer (Invitrogen)

250 mM Imidazole-HCl pH 6.4
60 mM $MgCl_2$
5 mM 2-mercaptoethanol
350 mM ADP

4.2.2 5 ml Formamide dye

4.9 ml	95 % formamide
0.01 g	0.2 % bromophenol blue
0.01 g	0.2 % xylene cyanol
0.1 ml	0.5 M EDTA pH8.0

4.3 Solutions for PCR

4.3.1 10X Standard *Taq* buffer (NEB)

100 mM Tris-HCl
500 mM KCl
15 mM $MgCl_2$
pH 8.3 @ 25°C

4.4 Solutions for Electrophoresis

4.4.1 1 L 6 % Acrylamide

57.15 g Acrylamide
2.85 g Bis-acrylamide
450.45 g Urea
200 ml 5X TBE
Ultra-pure water (up to 1 L)

4.4.2 1 L 10X TBE buffer

121.140 g Trizma base (MW = 121.14)
27.5 g Boric acid (MW = 61.83)
3.72 g EDTA diNa (MW = 372.24)
Ultra-pure water (up to 1 L)

4.5 Solutions for developing X-Ray film

4.5.1 2.5 L Developer

550 ml KODAK GBX Developer
1950 mlUltra-pure water
Mix by stirring or inverting bottle several times.
Store in a dark bottle

4.5.2 2.5 L Fixer

500 ml KODAK GBX Fixer
2000 mlUltra-pure water
Mix by stirring or inverting bottle several times.
Store in a dark bottle

5.0 DNA Marker for Gel Analysis

5.1 The DNA marker used for sizing of SSR alleles is a 100-330 base-pair AFLP ladder from Invitrogen USA.

5.2 Labeling of 100-330 bp AFLP ladder

5X Forward Exchange buffer (Invitrogen) = 2.0 ul T4 Polynucleotide Kinase (10 U/ul, NEB) = 2.0 ul
100-330 bp AFLP ladder (Invitrogen) = 4.0 ul
^{33}PdATP = 2.0 ul
Total reaction volume = 10.0 ul

5.3 Incubate at 37 ^{o}C for 10 -15 min. Heat treat at 65 ^{o}C for 10 min and chill on ice for 5 min. Dispense 10 ul TE buffer pH8.0 + 50 ul formamide dye. Store the labeled 100 - 330 bp AFLP ladder at 4 ^{o}C.

6.0 Procedure

6.1 (a) Dilute DNA samples to 50 ng/µl with TE buffer pH8.0. Store diluted DNA samples at -20 ^{o}C.

(b) Dilute the SSR primers as follows:

(i) Dilute the Forward (or Left) primer to 15 pmol (stock concentration of the Forward primer is 100 pmol).

(ii) Dilute the Reverse (or Right) primer to 5 pmol (stock concentration of

the Reverse primer is 100 pmol).

Store SSR primers at -20 °C.

6.2 Primer labelling

6.2.1 Primer labelling with ^{33}PdATP

(For 1 reaction)

Forward primer (15 pmol)	= 0.3 µl
Sterile ultra-pure water	= 0.4 µl
10X T4 Kinase Reaction buffer (NEB)	= 0.1 µl
T4 Polynucleotide Kinase (NEB)	= 0.1 µl
^{33}PdATP	= 0.1 µl
Total volume	= 1.0 µl

6.2.2 Incubate at 37 °C for 1.5 hrs. Heat treats the labeled primer at 70 °C for 10 min. Place tube on ice.

6.3 Polymerase Chain Reaction (PCR)

6.3.1 Arrange the 0.2 ml PCR tubes in a PCR tray.

6.3.2 Prepare Mix 1 in a 1.5 ml eppendorf tube as follows:

(For 1 reaction)

Sterile Ultra-pure water	= 5.7 µl
10X Standard *Taq* buffer (NEB)	= 1.0 µl
10 mM dNTP mix	= 0.2 µl
Taq Polymerase(5 U/µl, NEB)	= 0.1 µl
Total volume	= 7.0 µl

(Mix 1 should be prepared for the appropriate number of samples)
Place the tube on ice.

6.3.3 Set up PCR reactions as follows:

DNA (50 ng/µl)	= 1.0 µl
Reverse primer (5 pmol)	= 1.0 µl
Labelled Forward primer	= 1.0 µl
Mix 1	= 7.0 µl
Total volume	= 10.0 µl

6.3.4 PCR cycle employed in the SSR analysis

1 cycle	Pre-denature	95 °C	3 min
35 cycles	Denature Annealing Extension	95 °C (Depend on primer) 72 °C	30 sec 30 sec 30 sec
1 cycle	Final Extension Keep	72 °C 4 °C	5 min ∞

6.3.5 Store PCR products at -20 °C.

6.4 Electrophoresis

6.4.1 Casting of gel

(a) Clean both glass plates with 100 % Ethanol. Apply Rain Repellent on the short plate (32.5 x 39.5 cm).

(b) Place the 0.4 mm thick vinyl side spacers. Seal the glass plates with a rubber gasket.

(c) Measure 100 ml of 6 % acrylamide gel solution into a 100 ml glass beaker. Add 20 µl TEMED and 1ml of 0.1 % Ammonium persulphate.

(d) Pour the gel solution and then place the sharkstooth comb (flat surface facing inside). Let the gel solidify for about 3 - 4 hrs.

6.4.2 Gel Electrophoresis

(a) Clean the plate with water and remove the rubber gasket. Place the sharkstooth comb (flat surface facing outside).

(b) Clamp the plate to the Electrophoresis apparatus. Pour 1xTBE buffer into the upper and lower buffer chambers.

(c) Flush the wells and then load approximately 3 µl of Formamide dye.

(d) Pre-run the gel at 1600 V for 30 min.

(e) Denature the labeled 30-330 bp AFLP ladder at 70 ^{o}C for 5 min and put on ice.

(f) Add 10 µl of Formamide dye to the PCR product. Denature at 90 ^{o}C for 3 min and put on ice.

(g) Flush the wells with 1xTBE buffer and then load 5 - 6 µl samples. Run at 1600 V for 3.5 hrs.

(h) Transfer the gel onto a piece of 3MM Whatman paper and then dry the gel using gel dryer at 78 ^{o}C for 30 - 45 min.

(i) Place the dried gel onto an X-ray cassette.

(j) Expose X-ray film at -80 ^{o}C for 2-3 days.

(k) Develop the X-ray film in dark room as follows:

i) Developer 5 min
ii) Ultra-pure water 1 min
iii) Fixer 5 min
iv) Ultra-pure water 1 min

Recognition of alleles

The size of expected alleles is indicated in Table 1 and the expected profile is as in Figure 1 and 2.

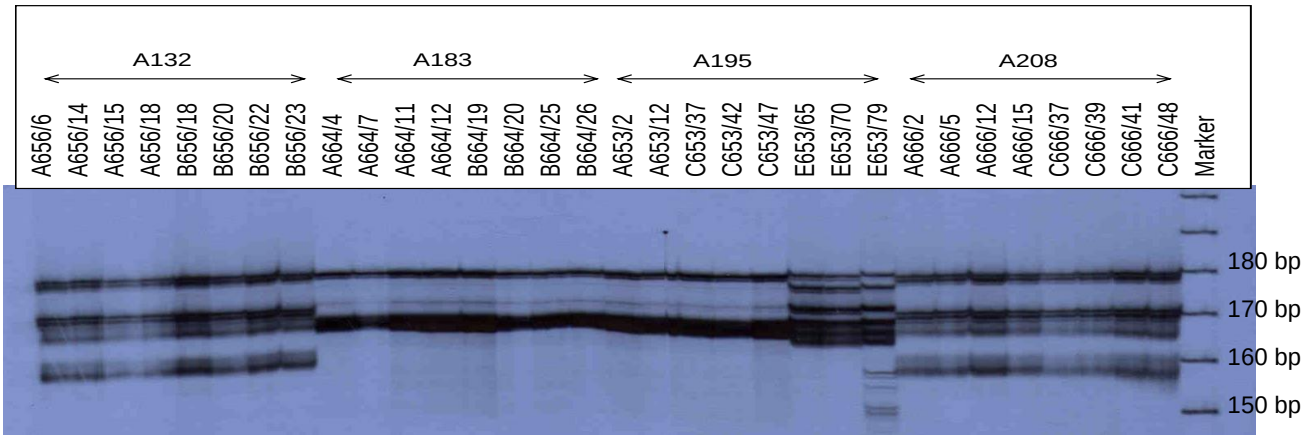

Figure 1: Profile for EAP03745 (sEg00097) using radioactive method (METHOD 1)

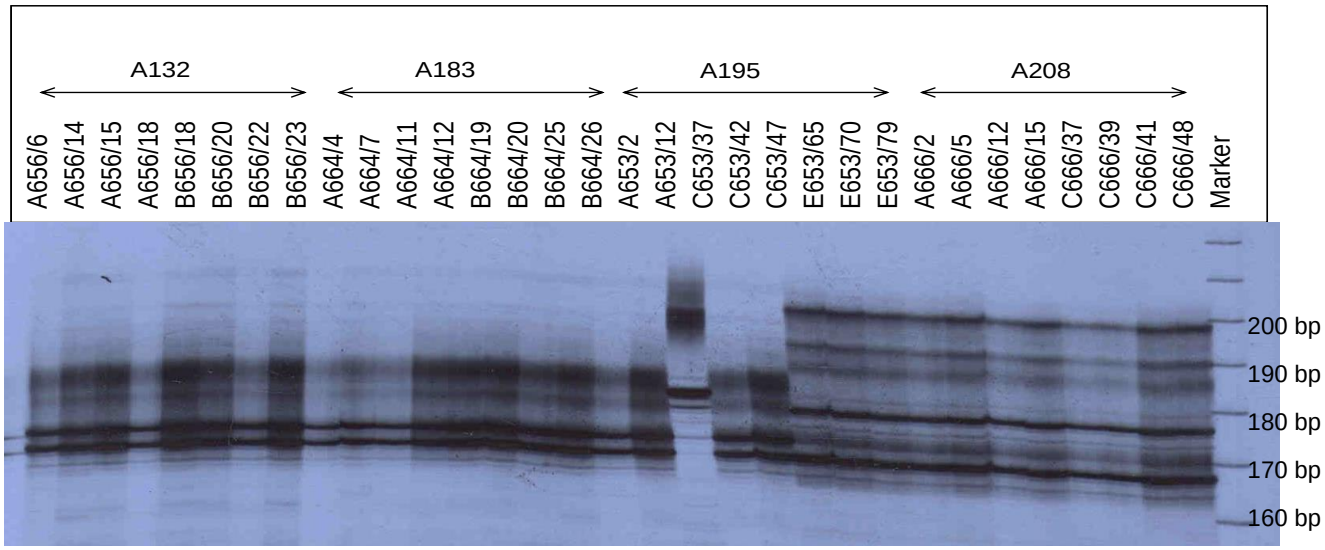

Figure 2: Profile for CNH01303 (sEg00038) using radioactive method (METHOD 1)

METHOD 2

SSR ANALYSIS USING LI-COR 4200 LONG READ NEN® GLOBAL EDITION IR2 SEQUENCER

1.0 Number of samples per test
Total 96 samples (48 samples at channel IR700 and IR800 separately)

2.0 Apparatus and equipment

2.1 PCR machine

2.2 Licor 4200 Sequencer

Components:
- 2.2.1 Computer
- 2.2.2 Global controller
- 2.2.3 Electrophoresis apparatus with upper buffer chamber, upper buffer tank lid, buffer chamber drain valve, silicone tubing gasket material, lower buffer tank lid, lower buffer tank,
- 2.2.4 High voltage cable
- 2.2.5 One pair of Starphire glass plates (25 cm wide, 0.5 cm thick): notched glass front plate and rectangular glass back plate
- 2.2.6 One pair of 0.25 mm thick side spacers
- 2.2.7 One piece of 0.25 mm thick sharkstooth combs (48 wells)
- 2.2.8 Left rail assembly and right rail assembly

2.3 Micropipettes (all range)

2.4 Refrigerated bench top centrifuge

3.0 Chemicals

All chemicals should be of 'Analytical Reagent' grade or better.

3.1 Chemicals required for primers

- 3.1.1 M-13 primer (5'- GGA AAC AGC TAT GAC CAT -3') labelled with IR-dye 700 and 800
- 3.1.2 M-13-tailed primers (forward)
- 3.1.3 Primer (reverse)
- 3.1.4 TE buffer pH8.0 (10 mM Tris-HCl, 0.1 mM EDTA)

3.2 Size Standards for LI-COR DNA analyzer

- 3.2.1 50-350 Size Standard IRdye labelled 700 and 800 (for range 50 – 350 bp)
- 3.2.2 50-700 Size Standard IRdye labelled 700 and 800(for range 50 – 700 bp)

3.3 Chemicals for Polymerase Chain Reaction (PCR)

- 3.3.1 TE buffer pH8.0 (10 mM Tris-HCl, 0.1 mM EDTA)
- 3.3.2 50 mM Magnesium Chloride (MgCl2)
- 3.3.3 10x PCR buffer
- 3.3.4 Ultra-pure water
- 3.3.5 10 mM dNTP mix
- 3.3.6 5 U/µl Taq DNA polymerase
- 3.3.7 50 mg/ml Bovine Serum Albumin

3.4 Chemicals for electrophoresis *(* The chemicals mentioned below should be of ultra-pure grade)*

3.4.1 95 % Ethanol
3.4.2 30 % Acrylamide/ Bisacrylamide (19:1)
3.4.3 Urea (Amresco)
3.4.4 Tris-HCl pH8.0
3.4.5 Boric acid
3.4.6 Na_2EDTA
3.4.7 Deionised water (≈18.0 MOhm)
3.4.8 N,N,N',N' Tetramethylethylenediamine (TEMED) - Add before use
3.4.9 Ammonium persulfate (APS) – add before use
3.4.10 1X and 10X Tris/Borate/EDTA (TBE) buffer
3.4.11 Loading dye

4.0 Solutions

4.1 Solutions for PCR

4.1.1 PCR buffer

500 mM	Potassium chloride (KCl)
100 mM	Tris-HCl pH 8.3
15 mM	Magnesium Chloride ($MgCl_2$)

4.2 Solutions for Electrophoresis

4.2.1 500 ml 5 % Acrylamide

75 ml	30 % Acrylamide/ Bisacrylamide (19:1) (Biorad)
45 ml	10X TBE
189 g	Urea

Ultra-pure water (up to 500 ml)

4.2.2 1 L 10X TBE buffer

121.140 g	Trizma base (MW = 121.14)
27.5 g	Boric acid (MW = 61.83)
3.72 g	EDTA diNa (MW = 372.24)

Ultra-pure water (up to 1 L)

4.2.3 5ml Bromophenol blue Dye
250 µl 0.5 mM EDTA pH8.0
100 mg Bromophenol blue
Sterile Ultra-pure water (up to 5 ml)

4.2.4

20 ml	Licor Loading buffer
15 ml	Deionized Formamide (Amresco, Commercially purchased)
5 ml	Bromophenol blue Dye

4.3 DNA Marker for Gel Analysis

The DNA markers used for the sizing of SSR alleles are 50 – 350 bp and 50 – 700 bp Sizing Standards labelled with IRDye infrared dye 700 nm and 800 nm from LI-COR USA.

5.0 Procedure

5.1 Polymerase Chain Reaction

5.1.1 Transfer 3 µl of 50 ng/µl DNA of each sample into a PCR plate.

5.1.2 Prepare PCR Mixture 1 below in a 1.5 ml eppendorf tube:

(For 1 reaction)

Ultra-pure water	= 8.30 µl
10X PCR buffer	= 1.50 µl
50 mM $MgCl_2$	= 0.60 µl
10 mM dNTP mix	= 0.30 µl
50 mg/ml BSA	= 0.06 µl
Taq Polymerase(5U/µl)	= 0.24 µl
Total volume	= 11.0 µl

(Prepare PCR Mixture 1 for the appropriate number of samples)

Place the tube on ice.

5.1.3 Set up PCR reactions as follows:
(For 1 reaction)

TE Buffer	= 0.30 µl
Reverse primer (100 pmol/ µl)	= 0.05 µl
M13-tailed Forward primer (100 pmol/ µl)	= 0.05 µl
IRD labelled- M13 primer (1 pmol/ µl)	= 0.60 µl
PCR Mixture 1	= 11.0 µl
DNA	= 3.0 µl
Total volume	= 15.0 µl

5.1.4 PCR programme employed in the SSR analysis

cycle	Pre-denature	95 °C	60 sec
35 cycles	Denature Annealing Extension	94 °C (Depends on primer) 72 °C	30 sec 60 sec 120 sec
1 cycle	Final Extension Hold	72 °C 10 °C	15 min ∞

5.1.5 Store PCR products at -20 °C.

5.2 Electrophoresis

5.2.1 Cleaning the plates

(a) Pour a small amount (6 cm circle) of 2 % DECON onto the side of the plate that will have contact with the gel.

(b) Work the solution into a lather with a sponge and thoroughly scrub the entire plate. Remove any dried-on polyacrylamide and rinse well with water.

(c) Pour distilled water onto the plate. Work the water around the plate with a gloved hand and watch for bubble formation from detergent residue. Repeat until no bubbles form.

(d) Repeat steps 1-3 above with the second plate.

(e) Rinse the plates with deionized distilled water. Stand plates in a rack to air dry.

Caution: *Always follow proper laboratory safety procedures. Always wear gloves and safety glasses when working with chemicals.*

5.2.2 Assembling the Gel Sandwich

(a) Assemble the gel sandwich according to the manufacturer's protocol.

5.2.3 Gel Preparation

(a) To prepare the polyacrylamide gel, the chemicals required are:
- i) 30 % Acrylamide–N,N'-methylene-bis (acrylamide) gel solution (Bio-Rad)
- ii) 1x and 10X Tris/Borate/EDTA (TBE) buffer
- iii) Urea (Amresco)
- iv) Deionised water (≈18.0 MOhm).
- v) N,N,N',N' Tetramethylethylenediamine (TEMED) - Add before use
- vi) Ammonium persulfate (APS) – add before use

** The chemicals mentioned above are of ultra-pure grade.*

(b) The following items are required to pour the gel:
- i) Comb
- ii) Pipette
- iii) Assembled gel sandwich

To prepare 150 ml of 5%gel solution

30 % Acrylamide-bis gel solution (Bio-Rad)	25 ml
10X Tris/Borate/EDTA (TBE) buffer	15 ml
Urea (Amresco)	63 g
Deionised water (≈18.0 MOhm)	Up to 150 ml

To prepare 20 ml ready-to-pour 5 % gel solution

5 % gel solution	20 ml
10 % Ammonium persulfate (APS)	150 µl
TEMED	15 µl

5.2.4 Pouring a 5 % Gel

(a) Measure and filter the solution using a membrane filtration system 20 ml of 5% acrylamide solution in 50 ml glass beaker. Set the gel solution to room temperature until use.

(b) Add 150 µl of 10% APS per 20 ml of gel solution and swirl gently. Add 15 µl TEMED just before pouring (pour within 3-5 minutes).

(c) Pour the gel solution at a steady rate avoiding any formation of air bubbles.

(d) Place the 0.25mm gel-casting comb upside down during polymerization to make a trough which forms the base of the wells, and is then inverted before loading the samples

(e) Let the gel solution polymerize for 1 to 1.5 hours.

(f) After the gel has polymerized, loosen the upper knob on each rail and remove the casting plate.

(g) Remove the comb. Clean and remove any excess gel inside of the back plate above the notched area where the comb was previously inserted using deionized water. Similarly, remove any gel from the outside of the plates, at the bottom and top of the gel sandwich, and next to the rails on the back plate.

(h) Use wipes and deionized water to clean the back and front plates, then 95% ethanol. The area on the plates (between the two bottom knobs) corresponding to the position of the sequencer's scanning window is the most important and should be carefully cleaned.

(i) If using a sharkstooth comb, re-insert the comb until the teeth just touch the gel. Hold the gel upright against a good light source in order to see the bottom of the well.

(j) Press the white rubber gasket into the recessed groove on the back of the upper buffer tank. Tighten the upper clamp knobs "finger tight". The electrophoresis apparatus is now fully assembled.

(k) Open the instrument door and place the lower buffer tank into position at the base of the heater plate. The tank has two recessed areas where the rails rest when the assembled gel apparatus is installed. The side of the lower buffer tank with the recessed areas is placed against the heater plate.

(l) Mount the gel apparatus on the instrument against the heater plate, with the bottom of the gel sandwich inside the lower buffer tank.

(m) Fill the upper buffer tank to Max Fill line with 1 x TBE and pour the remainder of the buffer into lower tank. (The level of buffer solution must be high enough to immerse the platinum electrodes)

(n) Place the upper and lower buffer tank lids onto the tanks. Insert the power cable on the upper buffer tank and connect it to the high voltage connector on the instrument chassis.

5.2.5 Starting Gel Runs

Pre-electrophoresis and electrophoresis using Saga^{GT} Automated Microsatellite Analysis Software.

(a) Start and log in to the Saga^{GT} Automated Microsatellite Analysis Software.

(b) Create a new project in Project Manager, or open the existing projects. Fill up project related information in DNA Source Manager, Molecular Weight Standards (MWS) Manager and Locus Manager.

(c) At the start of each gel, a gel template must be created that describes the DNA in sample lanes, the locus panel to use for each sample lane and the MWS panels used in the molecular weight standard lanes. (A new template can be created for each gel, but for increased efficiency, gel templates can be copied from saved templates or from gels that have already been run.)

(d) Start the pre-run. The pre-run time was set at 25 minutes and run time at 2 hours. Pre-set the electrophoresis conditions in the system as: voltage = 1500V, current = 40mA, power = 40W and Temperature = 45oC. (During the pre-run the microscope is focused and normal electrophoresis power is applied to the gel to remove impurities and create an even temperature across the gel.)

(e) Pre-runs are needed only for the first run on a gel. When reload a gel, the pre-run can be skipped by clicking “Reload” to start a gel, instead of clicking “Prerun”.

(f) At the end of the pre-run, the State in the Scanner Console window changes to “Load”.

5.2.6 Sample Loading

(a) Add Licor loading buffer (4.44) equal volume with PCR product and followed by denature samples and size standard (50-350bp) at 95°C for 3 minutes, immediately put the samples on ice and cover to reduce exposure to light.

(b) Open the instrument door and remove the upper buffer tank lid. Flush the wells with a 20cc syringe to remove any urea or other particulate matter. Be careful not to dislodge the teeth when flushing wells around the sharks tooth comb.

(c) Load the samples with the 8-channel pipette with flat 0.2 mm micropipette tips.

(d) Carefully position the tip(s) between the glass plates and slowly release the mixture into the wells. Load up to 0.8 – 1.0 µl per well depending on the PCR products.

(e) After sample loading, replace the upper buffer tank lid, close the instrument door, and start the run by clicking the “Run” button.

(f) After the gel has run the prescribed length of time, electrophoresis automatically stops and the gel images are automatically transferred from the instrument to the Saga Application Server for analysis.

5.2.7 Disassembly

(a) Remove the buffer tank lids and disconnecting the power cable, draining the buffer solution with the gum rubber tubing, while the apparatus is still secured to the instrument.

(b) Take the gel assembly off the sequencer and remove the upper buffer tank and rails. Rinse the rails, spacers, and comb, and allow to air dry.

(c) Dispose of the acrylamide within 1-2 hours after the run is complete. Put a paper towel on top of the plate with acrylamide gel, the acrylamide will adhere to it and can be lift up easily from the plates.

(d) Pour a small amount of 2% DECON detergent onto the side of the plate that has contact with the gel. Work the solution into a lather with a sponge and thoroughly scrub the entire plate.

(e) Remove any dried-on polyacrylamide. Rinse well with water.

6.0 Recognition of alleles

The size of expected alleles is indicated in Table 1 and the expected profile is as in Figure 3 and 4.

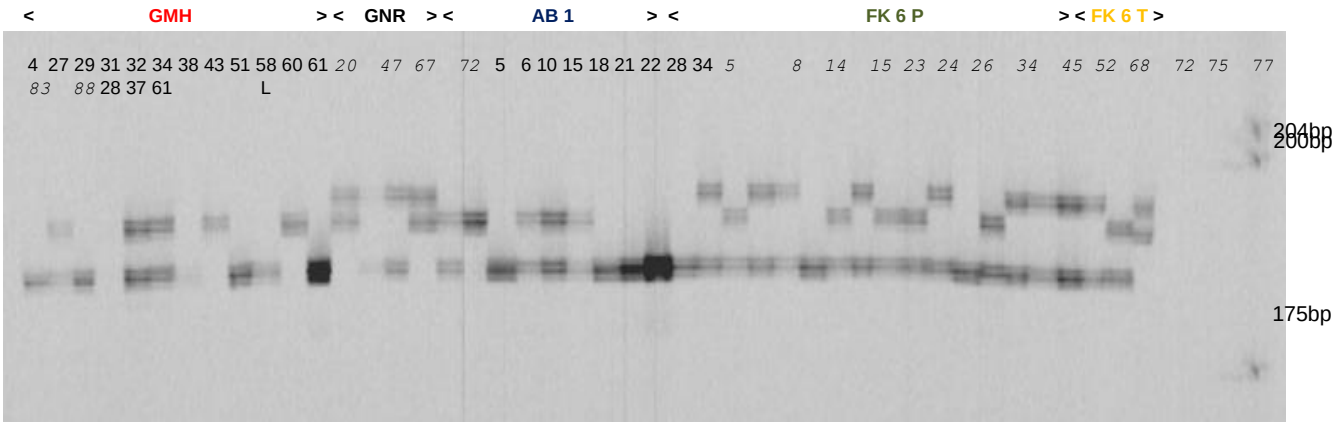

Figure 3: Expected Profile for EAP03745 (sEg00097) using LI-COR 4200 LONG READ IR SEQUENCER (METHOD 2)

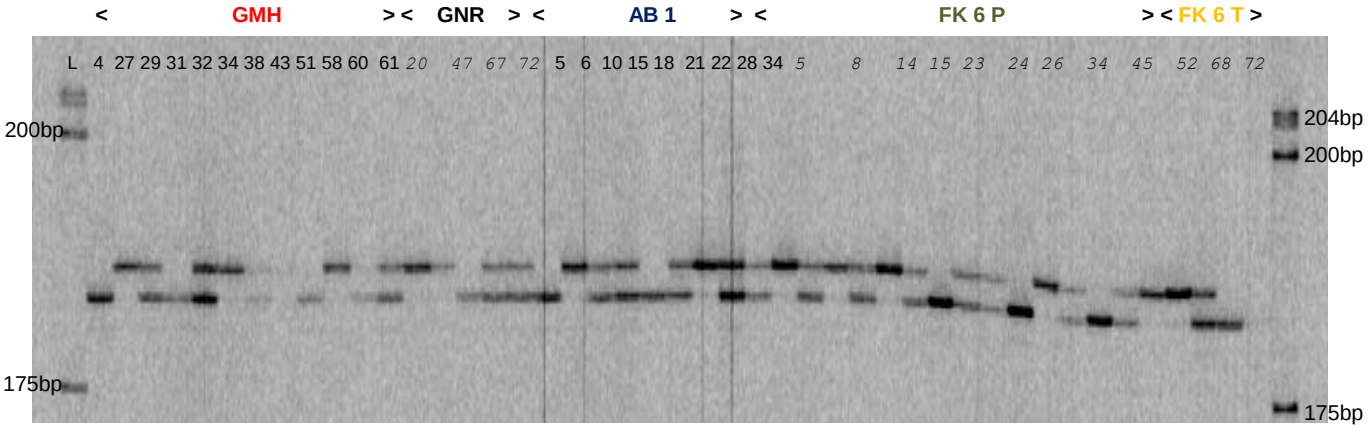

Figure 4: Expected Profile for CNH01303 (sEg00038) using LI-COR 4200 LONG READ IR SEQUENCER (METHOD 2)

Literature

Singh, R., Jayanthi, N., Tan, S-G., Jothi, M.P. and Cheah, S-C. 2007. Development of simple sequence repeat (SSR) markers for oil palm and their application in genetic mapping and fingerprinting of tissue culture clones. *Asia Pacific Journal of Molecular Biology and Biotechnology* 15(3): 121-131.

Seng, T.Y. and Faridah, Q.Z. 2006. DNA extraction from mature oil palm leaves. *Journal of Oil Palm Research* 18: 219-224

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