



JURUSAN AGROEKOTEKNOLOGI
FAKULTAS PERTANIAN
UNIVERSITAS MULAWARMAN



HITI Himpunan Ilmu Tanah Indonesia
(Soil Science Society of Indonesia)



SERTIFIKAT

No. 046/UN.17.3/JA/IV/2021

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Dengan Tema “Aplikasi Teknologi Molekular dalam Pemuliaan Tanaman”

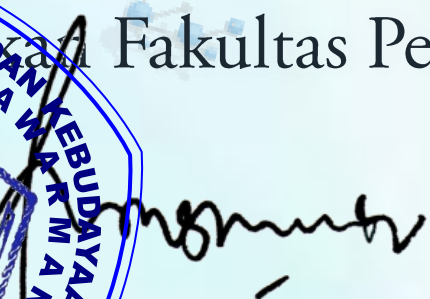
Samarinda, 10 April 2021

Dekan Fakultas Pertanian

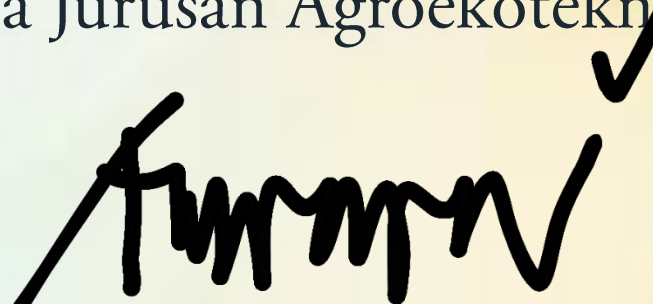
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Jurusan Agroekoteknologi, Fakultas Pertanian, UMMUL

ANALISIS KERAGAMAN GENETIK TANAMAN PISANG ASAL KALIMANTAN TIMUR MENGGUNAKAN MARKA MORFOLOGI DAN MOLEKULER

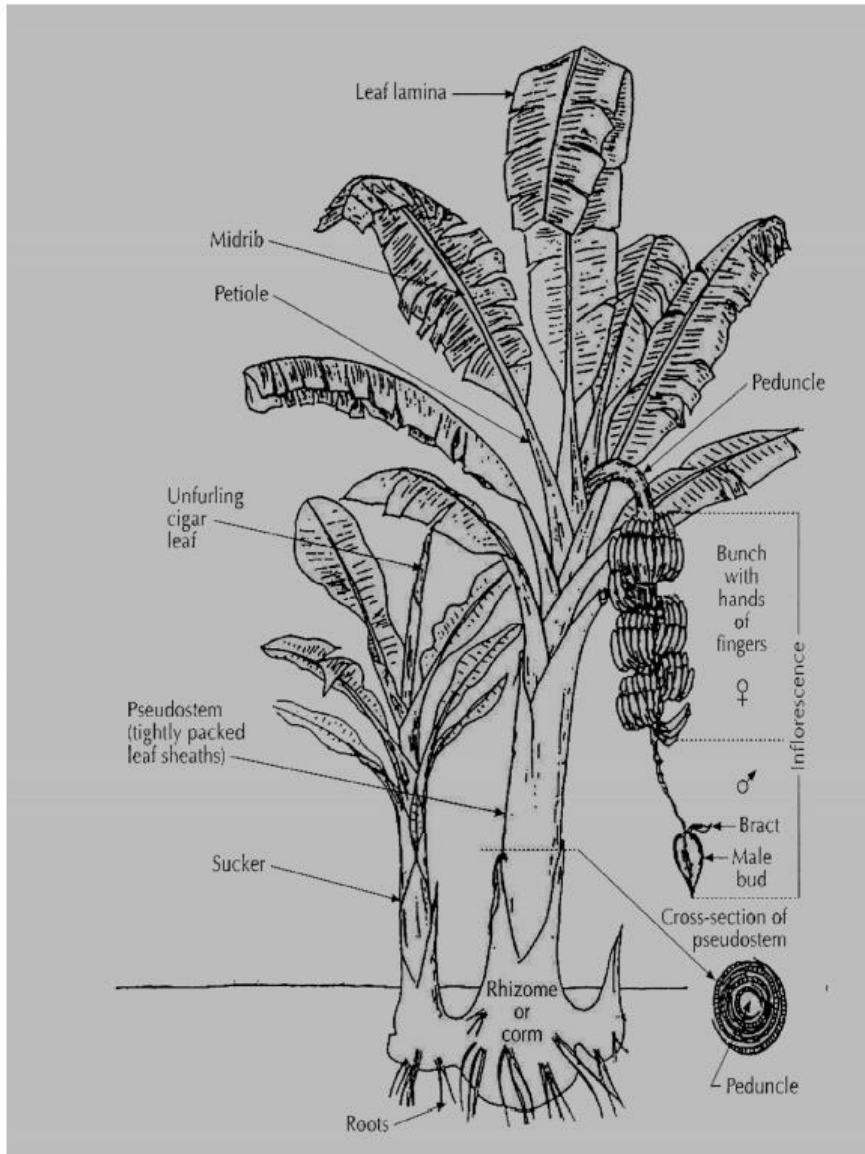
Widi Sunaryo, Ph.D



Universitas Mulawarman



Morfologi Pisang



Champion 1963; Jones 1999)

- **Leaf lamina** (lembaran daun)
- **Petiole** (tangkai Daun)
- **Cigar leaf** (daun yang masih menggulung)
- **Midrib** (tulang daun bagian tengah)
- **Pseudostem (batang semu)**
- **Sucker** (Anakan/ratoon)
- **Corm/Rhizome** (bonggol)
- **Inflorescence** (Bunga + buah)
- **Peduncle** (tangkai tandan)
- **Bunch** (Tandan; hands/sisir, Fruits buah, pedicle)/tangkai buah)
- **Rachis** (poros buah antara tandan dan jantung)
- **Male bud/jantung** (Male and female flower)
- **Bractea** (penutup bunga)
- **Flower anatomy** (stigma, anther, pollens, free tepal, compound tepal, ovary).



Kerabat Musa spp.

Jumlah Kromosom	Sub-Genus	Species	Penyebaran	Contoh
2n = 14	Ingentimusa	<i>Musa ingens</i>	Papua New Guinea, Papua	Giant Banana (30 m height)
2n = 20	Callimusa	15 species	Borneo, China, Indochina, Malaysia, Vietnam, Thailand	<i>Musa flavida</i> (Borneoensis)
	Australimusa	18 species	Maluku, Borneo, Philipina, Papua, Taiwan, Australia	Pisang Tongka Langit
2n = 22	Musa	<i>Musa acuminata</i>	Indonesia, IMalaysia, Thailand, Vietnam, China, India, Jepang, Amerika tengah	Pisang Monyet
		<i>Musa balbisiana</i>	Indonesia, IMalaysia, Thailand, Vietnam, China, India, Jepang, Amerika tengah	Pisang klutuk
		10 species	Indonesia, IMalaysia, Thailand, Vietnam, China, India, Jepang, Amerika tengah	<i>Musa halabanensis</i>
	Rhodoclamys	9 species	India, Burma, Bangladesh, Thailand, China, Vietnam	<i>Musa aurantiaca</i>

* Information in the table was compiled from a number of sources (Simmonds & Weatherup 1990; INIBAP 2000; Sharrock 2000; Constantine & Rossel 2001; Hakkinen & Sharrock 2001; Pollefeys et al. 2004; Ploetz et al. 2007)



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Kerabat Musa spp.

Musa ingens.



Pisang Raksasa 30 m (Papua)

Musa x troglodyatarum L.



Pisang Tongka langit (maluku)



LELUHUR PISANG MODERN

Musa x paradisiaca



Sunaryo et al. 2019

Musa acuminata

Diploid $2n=22$

Origin of dessert banana

Genome: AA

Contoh: pisang Monyet/Liar

Musa balbisiana

Diploid $2n=22$

Origin of cooking banana

Genome: BB

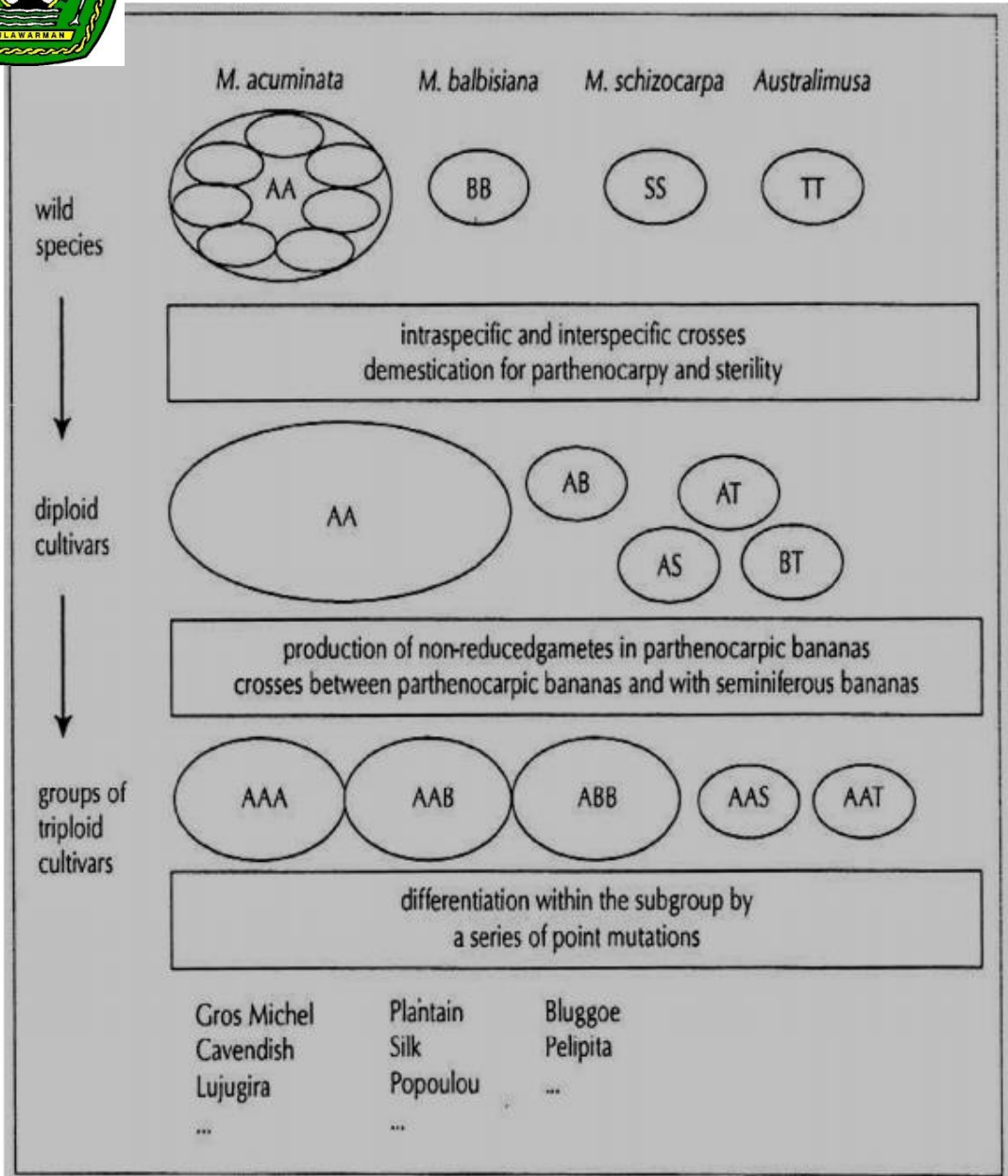
Contoh: pisang Klutuk



Variasi Genome Pisang Modern (*Musa x paradisiaca*)

Theoretical score	Ploidy Level		
	2x	3x	4x
15	AA (16–23)	AAA (15–21)	AAAA (15–20)
30			AAAB (27–35)
35		AAB (26–46)	
45	AB (46–49)		AABB (45–48)
55			
60		ABB (59–63)	ABBB (63–67)
75	BB (69)		

Simmonds and Shepherd, 1955



Banana Domestication



Persilangan Intra dan inter-spesifik
Hasil: *amphidiploid (AB)*



Persilangan intra/inter *amplidiploid partenocarpic* atau dengan pisang diploid normal



AAA, AAB, ABB, BBB,
AABB, AAAB, ABBB, BBBB
Pisang Modern



M. acuminata* vs *M. balbisiana



Pseudostem more or less heavily marked with brown or black blotches



Margin of the petiole canal erect or spreading, with scarious wings below, not clasping pseudostem



Peduncle usually downy or hairy



Short pedicels



Ratio (length to width) of bract shoulder usually high (ratio<0.28)



No or few blotches on the pseudostem



Margin of the petiole canal inclosed, not winged but clasping pseudostem



Peduncle glabrous



Long pedicels



Width/length ratio of bract shoulder usually low (ratio>0.30)



M.cuminata* vs *M balbisiana



Bracts reflex and roll back after opening



Bract shape lanceolate or narrowly ovate, tapering sharply from the shoulder



Bract apex acute



Bract scars prominent



Bracts do not reflex



Bract shape broadly ovate



Bract apex obtuse



Bract scars scarcely prominent



Simmonds and Shepherd's (1955) scoring system

No	Character	Musa acuminata	Musa balbisiana
1	Pseudostem colour	More or less heavily marked with brown or black blotches	Blotches very slight or absent
2	Petiole canal	Margin erect or spreading, with scarious wings below, not clasping pseudostem	Margin inclosed, not winged but clasping pseudostem
3	Peduncle	Usually downy or hairy	Glabrous
4	Pedicels	Short	Long
5	Ovules	Two regular rows in each loculus	Four irregular rows in each loculus
6	Bract shoulder	Usually high (ratio<0.28)	Usually low (ratio>0.30)
7	Bract curling	Bracts reflex and roll back after opening	Bracts do not reflex
8	Bract shape	Lanceolate or narrowly ovate, tapering sharply from the shoulder	Broadly ovate, not tapering sharply
9	Bract apex	Acute	Obtuse
10	Bract colour	Red, dull purple or yellow outside; pink, dull purple or yellow inside	Distinctive brownish-purple outside; bright crimson inside
11	Colour fading	Inside bract colour usually fades to yellow towards the base	Inside bract colour usually continuous to base
12	Bract scars	Prominent	Scarcely prominent
13	Free tepal of male flower	Variably corrugated below tip	Rarely corrugated
14	Male flower colour	Creamy white	Variably flushed with pink
15	Stigma colour	Orange or rich yellow	Cream, pale yellow or pale pink



Scoring Using Description Book (Inibab, 1996)

Descriptors for

Banana

(*Musa* spp.)

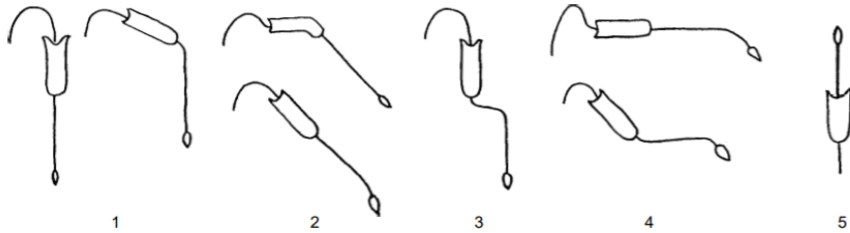


Fig. 8 Rachis position (adapted from De Langhe 1961)

★ 6.4.15 Male bud shape

Note the general shape of the male bud at harvest. (See Fig. 9)

- | | |
|----------------|-----------|
| 1 Like a top | 4 Ovoid |
| 2 Lanceolate | 5 Rounded |
| 3 Intermediate | |

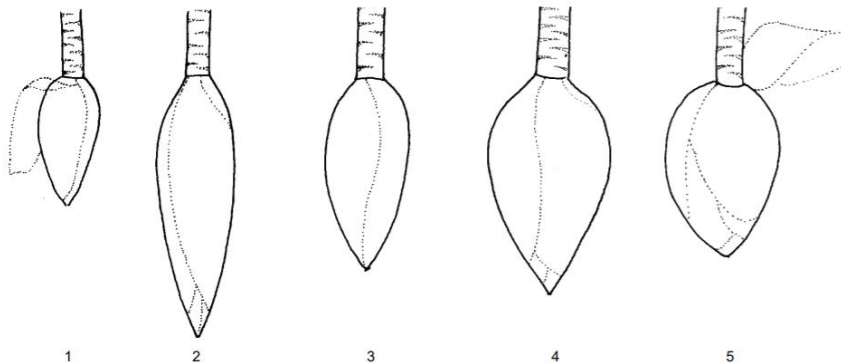


Fig. 9 Male bud shape

★ 6.3.3 Petiole canal leaf III

Leaf III is the third leaf counted from the last leaf (leaf I) produced before bunch emergence. Cut the petiole half way between the pseudostem and the leaf blade and examine the cross section. (See Figs. 4 and 6)

- 1 Open with margins spreading
- 2 Wide with erect margins
- 3 Straight with erect margins
- 4 Margins curved inward
- 5 Margins overlapping

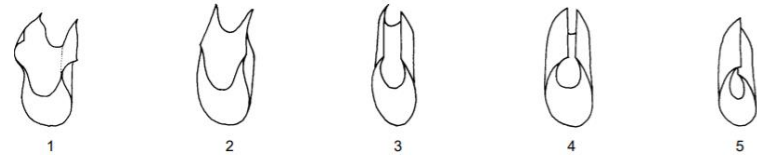


Fig. 6 Petiole canal leaf III

★ 6.5.2 Bract apex shape

Flatten the apex of the bract to observe its shape. (See Fig. 11)

(4.2.12)

- 1 Pointed
- 2 Slightly pointed
- 3 Intermediate
- 4 Obtuse
- 5 Obtuse and split

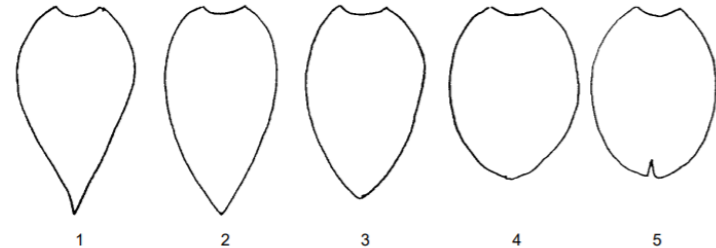


Fig. 11 Bract apex shape



Determinant scoring of genome group classification based on the morphological characters

Genome Group	Simmonds and Shepherd (1982)	Silayoi and Chamchalow (1987)	Sing et al (2014)	Rinaldi et al (2014)
AA/AAA	15-23	15-27	15-25	15-25
AAB	24-46	26-46	26-45	26-46
AB	49	-	46-49	47-58
ABB	59-63	59-63	59-65	59-63
ABBB	67	-	66-69	64-75
BB/BBB	-	70-75	70-75	-



Genome group scoring of six bananas from East Kalimantan

No .	Character	Talas	Rutai	Tanduk	Ambon	Liar/Mo nyet	Klutuk
1	Pseudostem colour	2	3	4	2	1	5
2	Petiole canal	1	1	5	1	1	5
3	Peduncle	2	2	5	2	1	5
4	Pedicels	5	4	5	1	1	5
5	Ovules	1	1	5	1	1	5
6	Bract shoulder	2	2	1	1	1	5
7	Bract curling	1	1	4	1	1	5
8	Bract shape	3	1	1	1	1	5
9	Bract apex	2	1	1	1	1	5
10	Bract colour	3	3	2	2	1	5
11	Colour fading	3	3	2	1	1	5
12	Bract scars	1	1	4	1	1	5
13	Free tepal of male flower	1	1	1	1	1	5
14	Male flower colour	2	1	4	2	1	5
15	Stigma colour	2	3	4	1	1	5
Total score		31	28	48	19	15	75



Genome group classification of six bananas from East Kalimantan

Banana cultivar	Total score	Genome classification
Liar/Monyet (<i>Musa acuminata</i>)	15	AA
Talas/Sunking	31	AAB
Rutai	28	AAB
Ambon	19	AAA
Tanduk	48	AB
Klutuk (<i>Musa balbisiana</i>)	75	BB

Sunaryo et al. 2019



Karakterisasi Morfologi



- A. Pisang Monyet/Liar
- B. Pisang Klutuk
- C. Pisang Talas/Sunking
- D. Pisang Rutai
- E. Pisang Ambon
- F. Pisang Tanduk



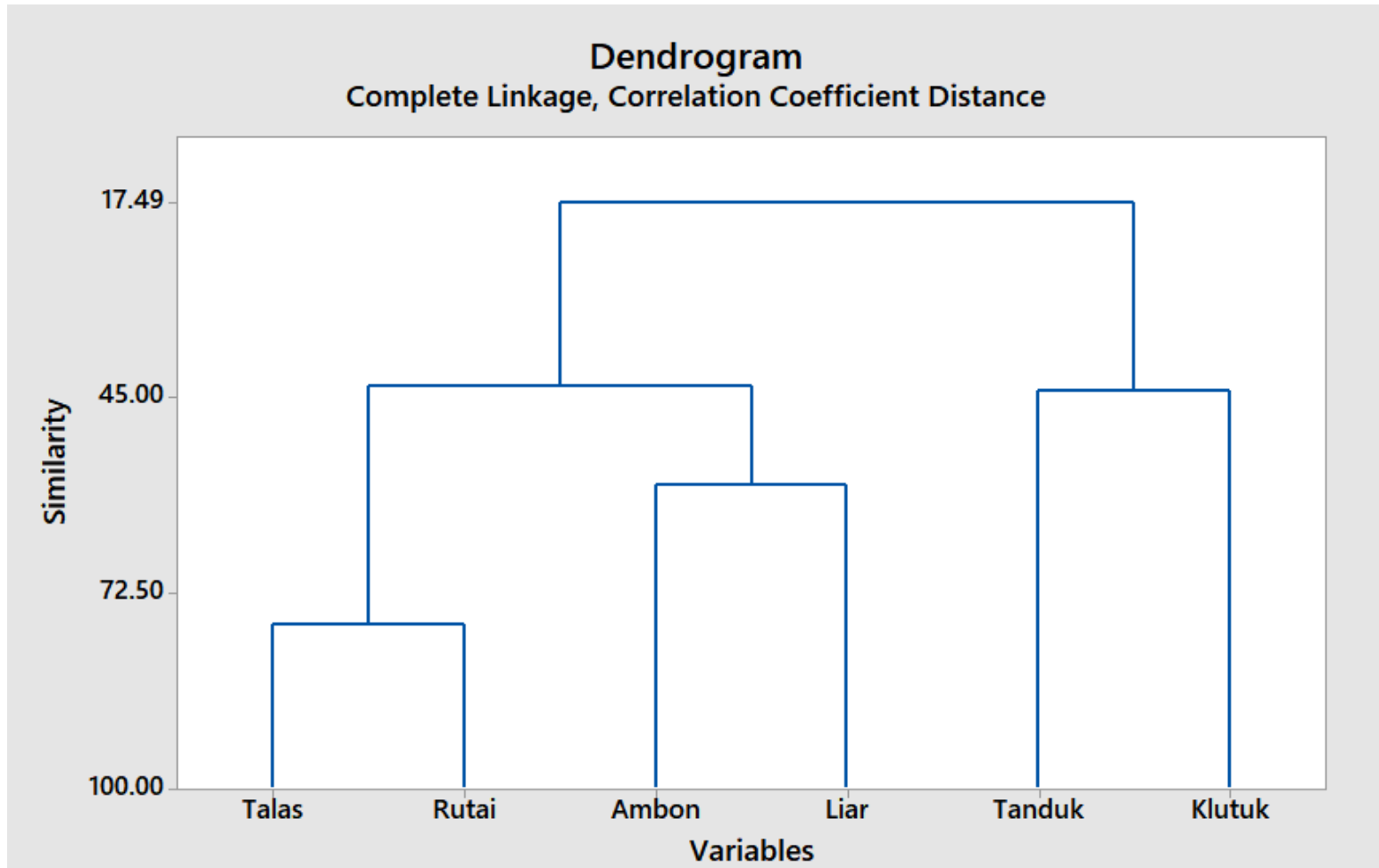
Karakterisasi Bunga Jantan



- A. Pisang Monyet/Liar
- B. Pisang Talas/Sunking
- C. Pisang Rutai
- D. Pisang Ambon
- E. Pisang Tanduk
- F. Pisang Klutuk



Kekerabatan genetik berdasarkan nilai Principal Component Analysis dikonversi dari 70 karakter terpilih berdasarkan Buku Deskriptor (Inibab 1996). Cluster analisis dilakukan menggunakan *MINITAB 17.1*





MARKA ISSR

- Inter simple sequence repeat (ISSR)-PCR adalah teknik yang melibatkan penggunaan sekuen mikrosatellite sebagai primer dalam reaksi PCR untuk menghasilkan penanda multilocus.
- Kelebihan: lebih sederhana, cepat dan sangat polimorfik.
- Manfaat: studi keragaman genetik, filogeni, penandaan gen, pemetaan genom dan biologi evolusioner.

Reddy et al. 2002



Obyek Penelitian

13 Varietas Pisang Asal Kalimantan Timur

No	Varietas	Nama Latin	Genom
1	Ambon	<i>Musa paradisiaca L</i>	AAA
2	Klutuk	<i>Musa balbisiana</i>	BB
3	Liar	<i>Musa acuminata</i>	AA
4	Mauli	<i>Musa paradisiaca L</i>	AAB
5	Raja	<i>Musa paradisiaca L</i>	AAB
6	Rutai	<i>Musa paradisiaca L</i>	AAB
7	Susu	<i>Musa paradisiaca L</i>	AAB
8	Awak	<i>Musa paradisiaca L</i>	ABB
9	Kepok	<i>Musa paradisiaca L</i>	ABB
10	Talas	<i>Musa paradisiaca L</i>	AAB
11	Kapas	<i>Musa paradisiaca L</i>	AA
12	Tanduk	<i>Musa paradisiaca L</i>	AB
13	Tembaga	<i>Musa paradisiaca L</i>	AAA

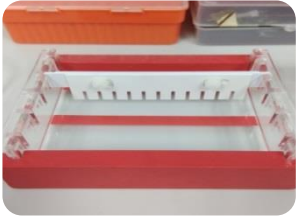


Primer ISSR

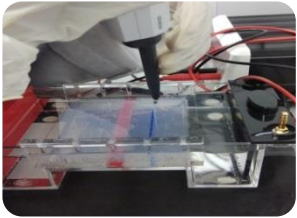
No.	Primer	Primer Sequens (5'-3')	Primer Length	Annealing Temperature (°C)
1	UBC 808	(AG)8C	53	47
2	UBC 811	(GA)8C	53	43.3
3	UBC 812	(GA)8A	47	45
4	UBC 818	(CA)8G	53	52
5	UBC 820	(GT)8T	47	45
6	UBC 825	(AC)8C	53	47
7	UBC 826	(AC)8T	47	49
8	UBC 834	(AG)8YT	44	50
9	UBC 835	(AG)8YC	50	50
10	UBC 840	(GA)8YT	44	53
11	UBC 841	(GA)8YC	50	46
12	UBC 843	(CT)8RA	44	47
13	UBC 848	(CA)8RG	50	51
14	UBC 855	(AC)8YT	44	60
15	UBC 868	(GAA)6	33	48



Metode



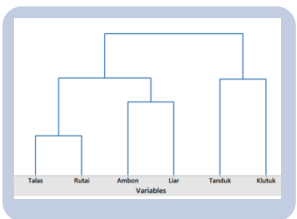
Isolasi DNA



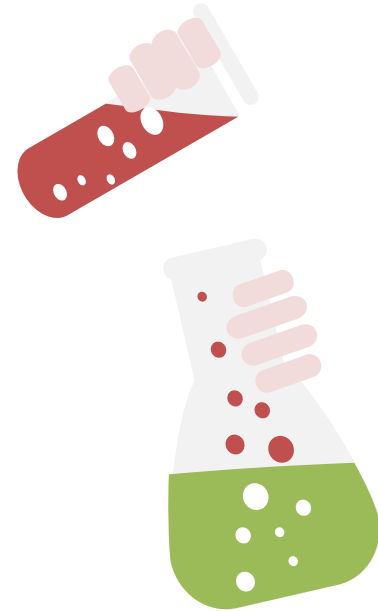
PCR



Electroforesis Gel dan Marker
Analysis: Polimorfisme etc

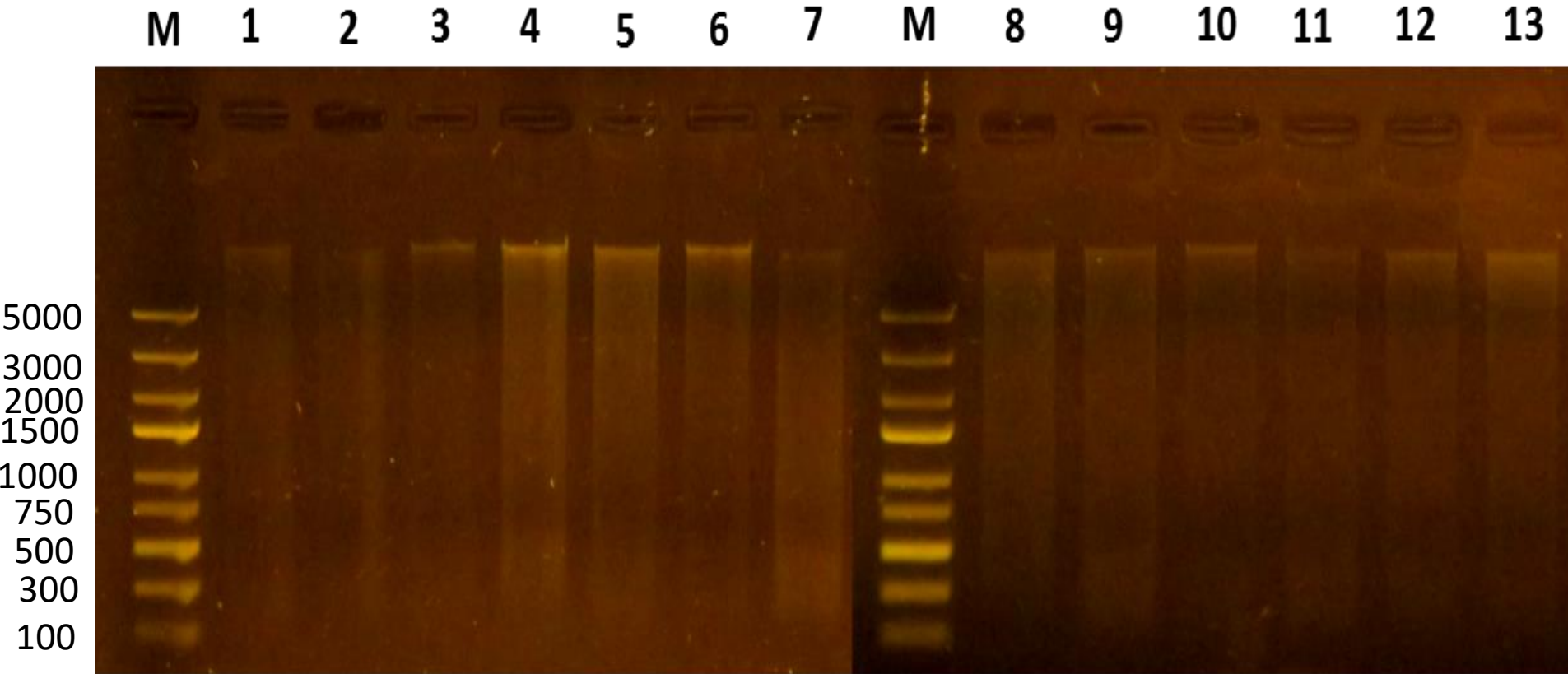


Cluster Analysis: NTYSIS





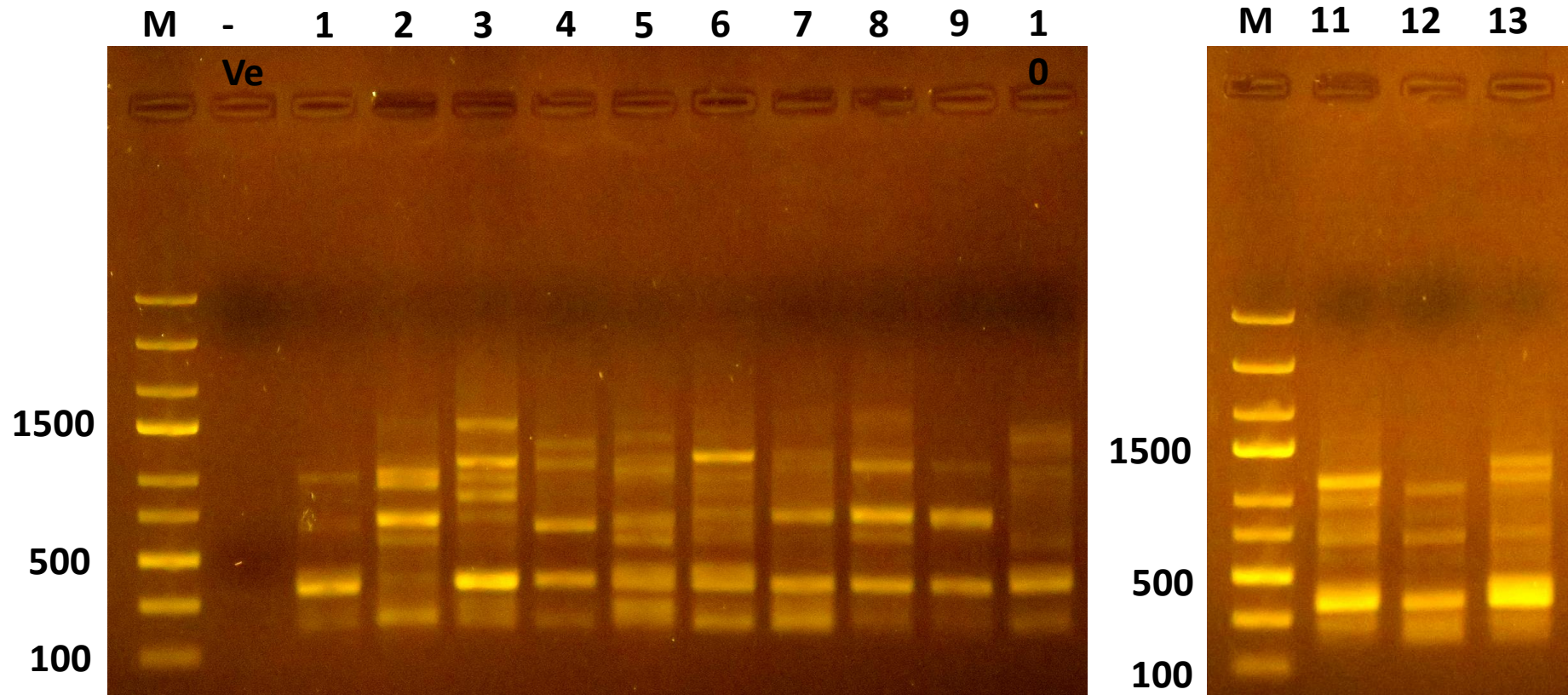
Hasil Isolasi DNA



Marker (M), Ambon (1), Klutuk (2), Liar (3), Mauli (4), Raja (5), Rutai (6), Susu (7), Awak (8), Kepok (9), Talas (10), Kapas (11), Tanduk (12), Tembaga (13).



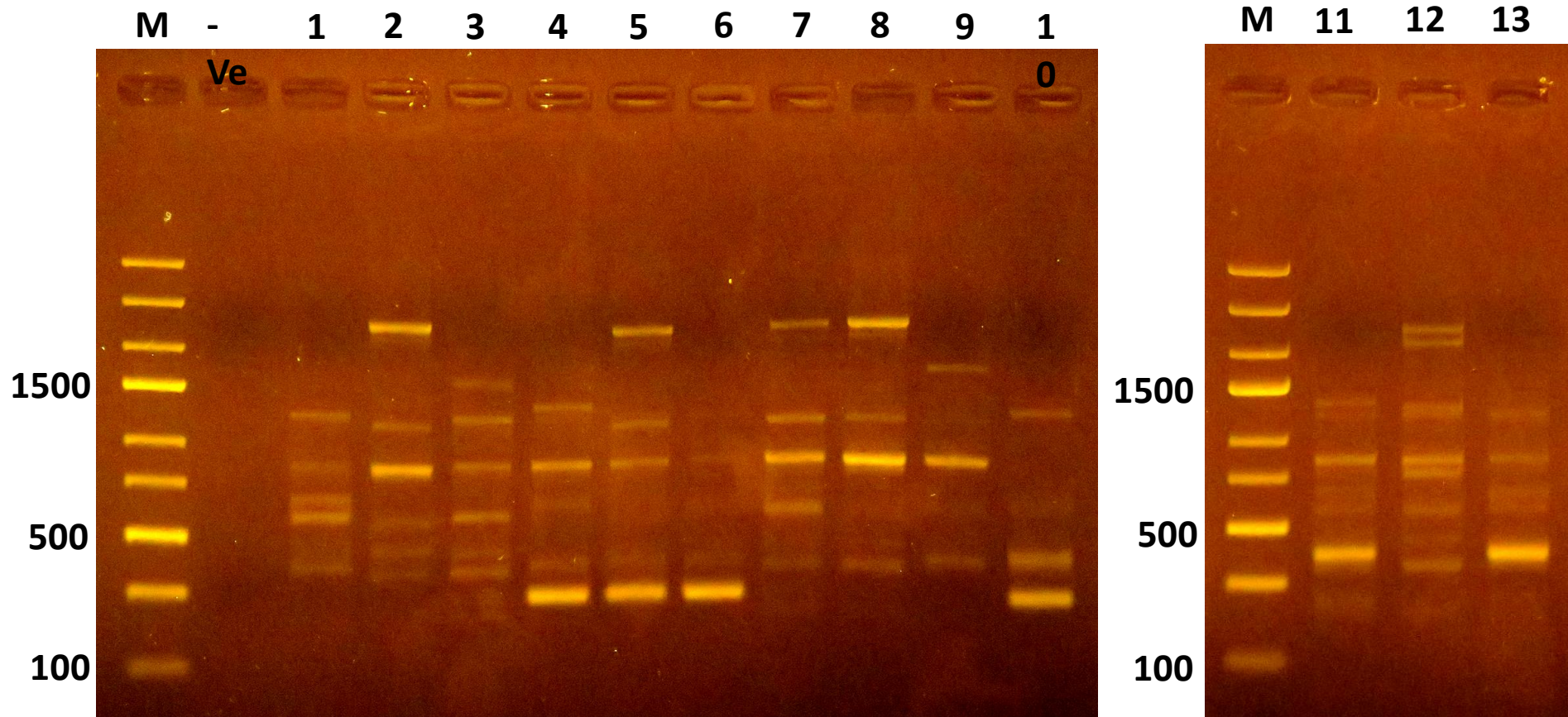
PCR Product using UBC 834



Marker (M), Ambon (1), Klutuk (2), Liar (3), Mauli (4), Raja (5), Rutai (6), Susu (7), Awak (8), Kepok (9), Talas (10), Kapas (11), Tanduk (12), Tembaga (13).



PCR Product using UBC 855



Marker (M), Ambon (1), Klutuk (2), Liar (3), Mauli (4), Raja (5), Rutai (6), Susu (7), Awak (8), Kepok (9), Talas (10), Kapas (11), Tanduk (12), Tembaga (13).



Mengubah Data Marker ISSR Data Biner

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	
1																
2	Primer	Locus	Ambon	Klutuk	Liar	Mauli	Raja	Rutai	Susu	Awak	Kepok	Talas	Kapas	Tanduk	Tembaga	T
3	UBC 808	L1	1	0	0	1	0	0	1	1	0	0	1	0	1	
4		L2	0	0	1	1	1	0	0	0	0	0	0	0	1	
5		L3	0	0	1	1	1	0	0	0	0	0	0	1	1	
6		L4	1	0	0	0	0	0	0	1	0	1	0	1	1	
7		L5	0	0	1	1	1	1	1	1	1	0	0	0	0	
8																
9	UBC 811	L1	0	0	1	0	0	1	1	1	0	0	0	0	1	
10		L2	1	0	1	1	1	1	1	1	1	1	1	1	1	
11		L3	0	1	0	0	0	1	1	0	0	0	1	0	0	
12		L4	0	1	0	0	0	0	0	1	0	0	0	1	0	
13		L5	1	0	0	0	0	0	0	0	1	0	1	0	1	
14		L6	1	1	0	1	1	0	1	1	0	1	0	0	0	
15		L7	0	0	0	0	1	0	0	0	0	0	0	1	0	
16		L8	1	0	0	1	0	0	0	0	0	0	0	0	0	
17		L9	0	0	1	0	0	0	0	0	0	0	0	0	0	
18		L10	0	1	0	0	0	0	0	1	1	0	0	0	0	
19		L11	0	0	0	0	0	0	0	0	0	1	0	0	0	
20		L12	0	0	0	0	0	0	0	0	0	1	0	0	0	
21		L13	0	0	0	1	0	0	0	0	0	0	0	0	0	
22																
23	UBC 812	L1	1	1	1	1	1	0	1	1	1	0	1	1	0	
24		L2	0	0	1	0	1	0	0	0	0	0	0	0	0	

1= Present, 0 = not present



Analisis Polimorfisme

No	Primer Code	Primer Sequence	Anneling Temperature (°C)	Amplified bands			
				Total	Monomorph	Polymorph	% Polymorphism
1	UBC 808	(AG) ₈ C	47	5	0	5	100
2	UBC 811	(GA) ₈ C	43.3	7	0	7	100
3	UBC 812	(GA) ₈ A	45	8	0	8	100
4	UBC 818	(CA) ₈ G	52	10	0	10	100
5	UBC 820	(GT) ₈ T	45	8	0	8	100
6	UBC 825	(AC) ₈ C	47	9	1	8	88.89
7	UBC 826	(AC) ₈ T	49	10	1	9	90
8	UBC 834	(AG) ₈ YT	50	9	2	7	77.78
9	UBC 835	(AG) ₈ YC	50	8	0	8	100
10	UBC 840	(GA) ₈ YT	53	0	0	0	0
11	UBC 841	(GA) ₈ YC	46	7	0	7	100
12	UBC 843	(CT) ₈ RA	47	8	0	8	100



Polymorphic Information Content (PCI), Effective Multiplex Ratio (EMR) dan Marker Index (MI)

No	Primer name	ML	PL	TL	PL%	PIC	EMR	MI
1	UBC 808	0	5	5	100	0.82	5.00	4.10
2	UBC 811	0	13	13	100	0.87	13.00	11.31
3	UBC 812	0	8	8	100	0.75	8.00	6.00
4	UBC 818	0	11	11	100	0.91	11.00	10.01
5	UBC 820	0	10	10	100	0.84	10.00	8.40
6	UBC 825	1	6	7	85.71	0.63	5.14	3.24
7	UBC 826	0	12	12	100	0.82	12.00	9.84
8	UBC 834	0	10	10	100	0.60	10.00	6.00
9	UBC 835	0	11	11	100	0.79	11.00	8.69
10	UBC 841	0	10	10	100	0.91	10.00	9.10
11	UBC 843	0	8	8	100	0.86	8.00	6.88
12	UBC 848	0	2	2	100	0.81	2.00	1.62
13	UBC 855	0	14	14	100	0.82	14.00	11.48
14	UBC 868	0	12	12	100	0.79	12.00	9.48
Total		1	132	133	1385.71	11.22	131.14	106.14
Average		0.07	9.43	9.5	98.98	0.80	9.37	7.58

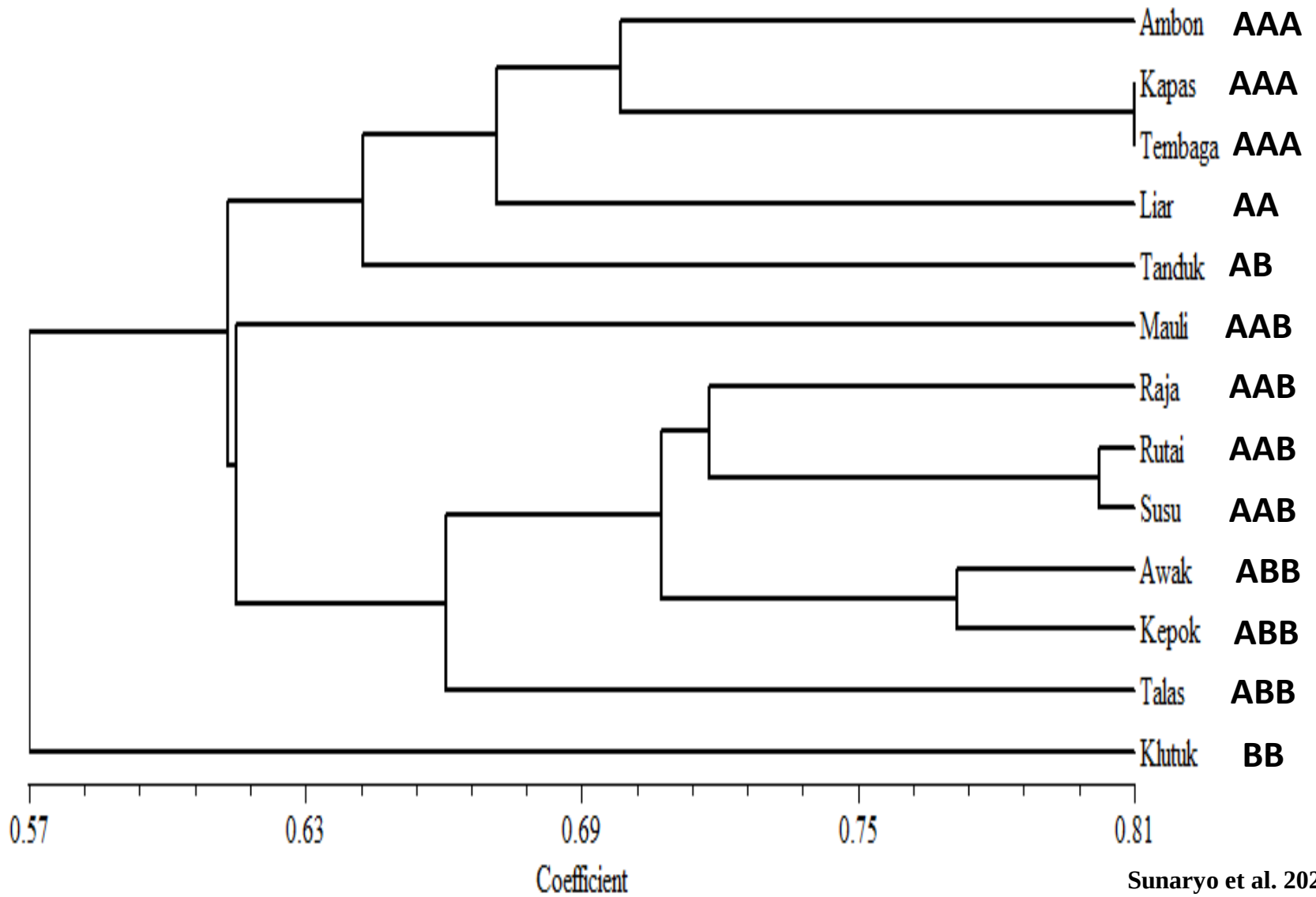


Similarity Analysis Based on ISSR Markers ; NTSYS

Varieties	Ambon	Klutuk	Liar	Mauli	Raja	Rutai	Susu	Awak	Kepok	Talas	Kapas	Tanduk	Tembaga
Ambon	1.00												
Klutuk	0.60	1.00											
Liar	0.69	0.53	1.00										
Mauli	0.64	0.42	0.64	1.00									
Raja	0.69	0.63	0.66	0.66	1.00								
Rutai	0.68	0.58	0.63	0.67	0.73	1.00							
Susu	0.69	0.61	0.62	0.63	0.71	0.80	1.00						
Awak	0.69	0.61	0.56	0.61	0.68	0.69	0.71	1.00					
Kepok	0.71	0.61	0.60	0.61	0.71	0.68	0.68	0.77	1.00				
Talas	0.65	0.55	0.51	0.52	0.65	0.66	0.69	0.62	0.68	1.00			
Kapas	0.73	0.60	0.69	0.59	0.56	0.69	0.63	0.60	0.63	0.63	1.00		
Tanduk	0.62	0.53	0.63	0.58	0.62	0.63	0.63	0.59	0.57	0.56	0.66	1.00	
Tembaga	0.66	0.58	0.63	0.60	0.54	0.62	0.57	0.56	0.56	0.56	0.81	0.66	1.00



Cluster analysis Based on ISSR Markers

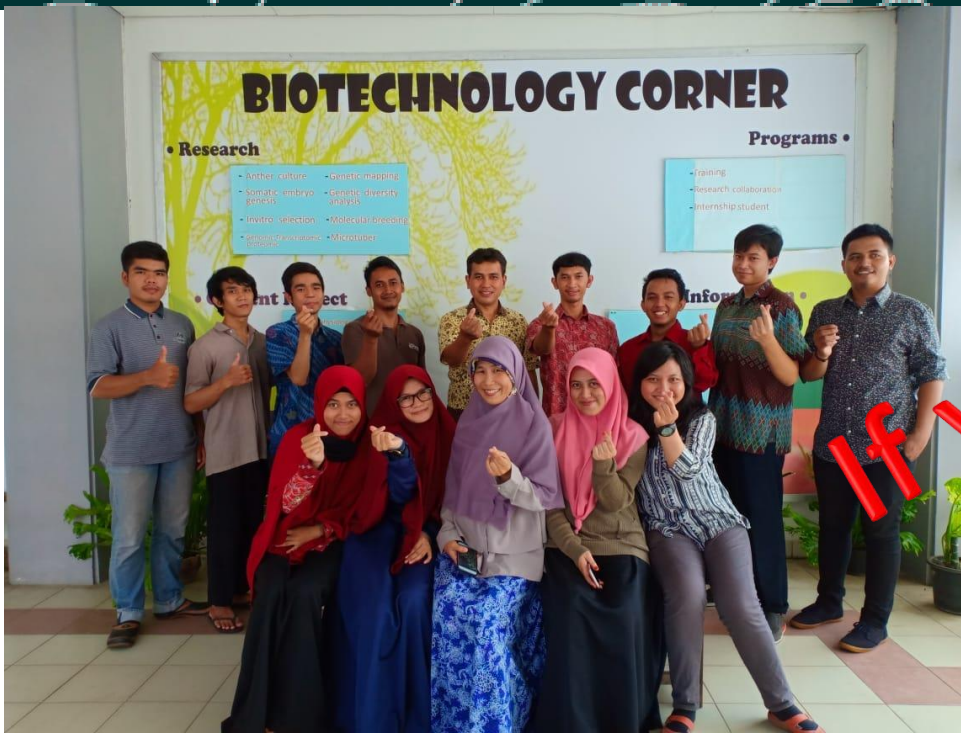




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THANKYOU

Special thanks for: WAHIDA, AHMAD MULYADI, RIZA WIDIATY, AND ANANDA N PRATAMA



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