

# Durian Marker Kit for Durian (*Durio zibethinus* Murr.) Identity

Emma K. Sales

**Abstract**—Durian is the flagship fruit of Mindanao and there is an abundance of several cultivars with many confusing identities/names.

The project was conducted to develop procedure for reliable and rapid detection and sorting of durian planting materials. Moreover, it is also aimed to establish specific genetic or DNA markers for routine testing and authentication of durian cultivars in question.

The project developed molecular procedures for routine testing. SSR primers were also screened and identified for their utility in discriminating durian cultivars collected.

Results of the study showed the following accomplishments:

1. Twenty (29) SSR primers were selected and identified based on their ability to discriminate durian cultivars,
2. Optimized and established standard procedure for identification and authentication of Durian cultivars
3. Genetic profile of durian is now available at Biotech Unit

Our results demonstrate the relevance of using molecular techniques in evaluating and identifying durian clones. The most polymorphic primers tested in this study could be useful tools for detecting variation even at the early stage of the plant especially for commercial purposes. The process developed combines the efficiency of the microsatellites development process with the optimization of non-radioactive detection process resulting in a user-friendly protocol that can be performed in two (2) weeks and easily incorporated into laboratories about to start microsatellite development projects. This can be of great importance to extend microsatellite analyses to other crop species where minimal genetic information is currently available. With this, the University can now be a service laboratory for routine testing and authentication of durian clones.

**Keywords**—DNA, SSR Analysis, genotype, genetic diversity, cultivars.

## I. INTRODUCTION

**D**URIAN, (*Durio zibethinus*) is tagged and revered in Southeastern Asia as the king of fruit [1] and is the flagship fruit in Mindanao. Moreover, among durian eaters it is known as a fruit that “smells like hell but tastes like heaven” due to its unusual odor. It is distinctive for its large size, unique odor and formidable thorn-covered hush. Southern Mindanao is considered the durian republic where several collection and cultivars are widely grown. Moreover, it grows almost exclusive in Mindanao and very few in other parts of the country.

This fruit is becoming more important and the demand had increased from year to year [1]. In the past, durian fruit is priced by piece but recently it is sold by the kilogram. This is

due to the increasing demand as fresh fruit as well as other uses such as flavor base of sweet edibles e.g. ice cream, candy, jam, preserve, etc. Interestingly, previous non durian eaters had acquired the taste for this fruit such that the demand spread from Mindanao to other parts of the country. Meanwhile, overseas consumers prefer durians that do not smell, taste sweet, slightly bitter, thick fruit meat and yellow fruit meat [2].

There are numerous varieties of durian. Some are considered with superior quality. Different market segments showed various consumer preferences and its distinction for superior quality depends on the consumer preferences like flavor, odor and meat/flesh content.

In view of the numerous cultivars available, preferences for recommended varieties fetch higher price as planting materials or as fresh produce. This therefore results to a greater demand for quality planting materials of specified variety. However, many cultivar names are thought to be synonyms for the same cultivars which in turn cause confusion among breeders and farmers. So far, no critical method for cultivar identification currently exist thus one of the constraints and gap is the lack of a proper and reliable identification of clones and varieties in the nursery. Identification is mostly done through evaluation of fruit and leaf traits. Varieties or clones should be properly identified as true to type before they are disposed to the farmers for planting. Any simple mistake or mislabeling will seriously jeopardize the economic profitability of the plantation. Mother plants/seedlings and other propagates used as source of planting materials should be checked, verified or confirmed to be the best variety (variety of interest) for multiplication.

With the advent of DNA technology, this gap can be bridged. Molecular marker techniques have proved useful in population and improvement studies, clone/genotype identification, certification of controlled species and hybrids, paternity determination, marker assisted early selection and development of strategies for improvement and suitable management of genetic resources. Moreover, genetic diversity of durian cultivars in the Philippines has been successfully assessed [3].

At present USM has an existing collection of 23 varieties of fruit bearing durian at the USM clonal garden. So far, characterization even at the morphological level has not been done. Identification is only through the difference in fruit appearance which is quite arbitrary.

Likewise, the university is also endowed with an established genetics and molecular biology laboratory equipped with the required facilities (thru the DA-BAR IDG

support) to ensure quality outputs. Up to the present, works on durian characterization remain very few. In Indonesia and in Malaysia, evaluation and characterization was undertaken via morphological and RFLP and RAPD markers [1]. In the Philippines, however, evaluation and is nil with the foregoing, this research endeavor is proposed.

## II. MATERIALS AND METHODS

### A. Plant Materials and DNA Isolation

Young leaves of all existing durian collections from USM genebank and different provinces of Regions IX, XI, XII and ARMM were collected and stored in a closed, ice-filled container to ensure the freshness of the samples prior to DNA extraction. DNA extraction protocol developed and optimized by USMARC Genetics and Molecular Biology was employed (unpublished result).

### B. Molecular Methods of Assessment

SSR marker techniques were employed to evaluate its utility in durian characterization. Additional primers were also screened using the SSR primers for cotton.

A total of 127 SSR primers were screened to determine its utility for durian identification and classification. The detailed procedures are as follows: Amplification of SSRs was performed using protocol adapted and modified by USM Mole Bio laboratory. Gel electrophoresis was done to visualize the band yield of the PCR done.

All primers yielding amplification were identified and selected based on intensity, resolution of bands, and number of bands produced. Those primers yielding at least 3 to 5 amplification products of good intensity and resolution were selected for further replicated trials using different durian varieties. Those primers revealing polymorphic patterns were determined and the extracted DNA data were subsequently analyzed statistically using the NTSYS pc software package version 2.01 [1]. A true dendrogram was constructed based on the matrix of similarity using the Unweighted Pair Group Method of Arithmetic Average (UPGMA) [4]. Specifically, results of PCR amplification using specific primers were scored as presence (1) or absence (0) of the amplified fragment [5]. Genetic similarity was evaluated using the unweighted pair grouping with arithmetic average (UPGMA) [6]. A dendrogram of genetic similarity were generated with the NTSYSPc Version 2.10z (1986-2002 Applied Biostatistics, Inc.).

The resolving power of the SSR primers, expressed as Polymorphism Information Content (PIC) was calculated using:

$$PIC = 1 - \sum_{i=1}^n P_i^2$$

where:  $P_i$  = frequency of the  $i$ th allele;  $n$  = number of alleles.

To determine specific primers that can discriminate a durian cultivar over that of other cultivars, band profiles generated per primer per cultivar were closely scrutinized. A band or molecular marker that are unique for particular cultivar were

selected and use as the marker for such cultivar. To ascertain the accuracy of the results, all experiments were done in triplicates.

Furthermore, a Standard allele for durian was developed and used as a reference for all the succeeding SSR analysis. This was done by putting together all the unique alleles generated from a set of SSR primers. Such unique alleles that were generated were named in reference to DNA fragments using a molecular wt marker (e.g. Phi x 174, HinfI). Allele name assignments were done based on its molecular size.

A summary of the procedure is as follows:

- 1) Screening of SSR primer/design and development of SSR primers for durian
- 2) Identification and selection of useful primers
- 3) Confirmation of optimized protocols and development of marker kit
- 4) Evaluation of primers used
- 5) Development of standard/reference alleles for durian for authentication/ identification.
- 6) One on one correspondence between a molecular marker vis a vis durian variety.

## III. RESULTS - MOLECULAR EVALUATION

Table I shows the durian varieties/accessions from different locations where young leaves were collected. DNAs were extracted from the said samples for DNA Analysis.

TABLE I  
LIST OF DURIAN CULTIVARS COLLECTED FROM DIFFERENT LOCATIONS

<i>Durian Cultivars /Accessions collected</i>	<i>Location</i>
1 Native Banga	Banga, South Cotabato
2 Native 1 Tampakan	Tampakan, South Cotabato
3 Native Sitio Milagrosa Tampakan	Sitio Milagrosa Tampakan, South Cotabato
4 Native Polomolok Farm 1	Polomolok Farm 1, South Cotabato
5 Native 2 Tampakan	Tampakan, South Cotabato
6 Native 3 Tampakan	Tampakan, South, Cotabato
7 Native Tupi Seed Farm (Bark)	Tupi Seed Farm, South Cotabato
8 Native Tupi Seed Farm (Duplicate)	Tupi Seed Farm, South Cotabato
9 Native Banga	Banga, South Cotabato
10 Arancillo Yolanda Farm Tupi	Tupi Yolanda Farm, South Cotabato
11 Arancillo Tupi Seed Farm	Tupi Seed Farm, South Cotabato
12 Arancillo Banga	Banga, South Cotabato
13 Chanee Polomolok	Polomolok, South Cotabato
14 Chanee Yolanda Farm 1 Tupi	Tupi Yolanda Farm1, South Cotabato
15 Puyat Polomolok Farm 1	Polomolok Farm 1, South Cotabato
16 Puyat Tupi Seed Farm	Tupi Seed Farm, South Cotabato
17 Cobb Banga	Banga, South Cotabato
18 Monthong Tupi Seed Farm	Tupi Seed Farm, South Cotabato
19 Unidentified Polomolok	Polomolok, South Cotabato
20 Chanee 3 Yolanda Farm 2 Tupi	Yolanda Farm 2 Tupi, South Cotabato
21 Suspected Duyaya A Banga	Banga, South Cotabato
22 Suspected Duyaya B Banga	Banga, South Cotabato
23 Native Polomolok Farm 2	Polomolok Farm 2, South Cotabato
24 Suspected Nanam Polomolok	Polomolok, South Cotabato
25 Suspected Nanam 4 Polomolok	Polomolok, South Cotabato
26 Suspected Nanam 1	Polomolok, South Cotabato
27 Polomolok Suspected	Polomolok, South Cotabato
28 Nanam 3 Polomolok	Polomolok, South Cotabato

<i>Durian Cultivars /Accessions collected</i>		<i>Location</i>	<i>Durian Cultivars /Accessions collected</i>		<i>Location</i>
29	Suspected Monthong Polomolok	Polomolok, South Cotabato	92	Native 5 Squibel Farm Kulaman	Kulaman Squibel Farm, Sultan Kudarat
30	Solomon Polomolok	Polomolok, South Cotabato	93	Native 9 Idio Fram Kulaman	Kulaman Idio Farm, Sultan Kudarat
31	Unidentified 2 Line 1 Polomolok	Polomolok, South Cotabato	94	Native 10 Idio Farm Kulaman	Kulaman Idio Farm, Sultan Kudarat
32	Unidentified 1 Polomolok	Polomolok, South Cotabato	95	Native 11 Idio Farm Kulaman	Kulaman Idio Farm, Sultan Kudarat
33	Unidentified 3 L3T3 Polomolok	Polomolok, South Cotabato	96	Native 1 Masilim Farm Kulaman	Kulaman Masilim Farm, Sultan Kudarat
34	D-24 Tupi Seed Farm	Tupi Seed Farm, South Cotabato	97	Native 12 Idio Farm Kulaman	Kulaman Idio Farm, Sultan Kudarat
35	L2 T1 Polomolok	Polomolok, South Cotabato	98	Arancillo Kulaman	Kulaman, Sultan Kudarat
36	Red Prun Tupi Seed Farm	Tupi Seed Farm, South Cotabato	99	Chanee Masilim Farm Kulaman	Kulaman Masilim Farm, Sultan Kudarat
37	Unidentified Tupi Seed Farm	Tupi Seed Farm, South Cotabato,	100	Chanee Kulaman	Kulaman, Sultan Kudarat
38	Tupi Seed Farm 2	Tupi Seed Farm 2	101	Puyat Idio Farm Kulaman	Kulaman Idio Farm, Sultan Kudarat
39	Native Old Makilala	Makilala, North Cotabato	102	Puyat Kulaman	Kulaman, Sultan Kudarat
40	Arancillo USM	USM, Kabacan, North Cotabato	103	D101 Mahinay Farm Kulaman	Kulaman Mahinay Farm, Sultan Kudarat
41	Arancillo B Balindog	Balindog, Kidapawan City	104	Monthong Kulaman	Kulaman, Sultan Kudarat
42	Arancillo C Balindog	Balindog, Kidapawan City	105	Mdur Kulaman	Kulaman, Sultan Kudarat
43	Chanee USM	USM, Kabacan, North Cotabato	106	Chanee Idio Farm Kulaman	Kulaman Idio Farm, Sultan Kudarat
44	Puyat USM	USM, Kabacan, North Cotabato	107	Davao Selection Masilim Farm Kulaman	Kulaman Masilim Farm, Sultan Kudarat
45	Puyat 2 Makilala	Makilala, North Cotabato	108	Unidentified 1 Kulaman	Kulaman, Sultan Kudarat
46	Puyat Makilala	Makilala, North Cotabato	109	Unidentified 2 Kulaman	Kulaman, Sultan Kudarat
47	D 101 USM	USM, Kabacan, North Cotabato	110	Unidentified 3 Kulaman	Kulaman, Sultan Kudarat
48	Cobb 1 USM	USM, Kabacan, North Cotabato	111	Unidentified 4 Kulaman	Kulaman, Sultan Kudarat
49	Cobb Makilala	Makilala, North Cotabato	112	Unidentified 5 Kulaman	Kulaman, Sultan Kudarat
50	Monthong USM	USM, Kabacan, North Cotabato	113	Arancillo BPI	BPI Bago Oshiro, Davao City
51	Alcon Fancy USM	USM, Kabacan, North Cotabato	114	Chanee 2815 BPI	BPI Bago Oshiro, Davao City
52	Alcon Fancy Makilala	Makilala, North Cotabato	115	Chanee BPI	BPI Bago Oshiro, Davao City
53	M Durr 88 USM	USM, Kabacan, North Cotabato	116	D 101 A BPI	BPI Bago Oshiro, Davao City
54	Umali Monthong USM	USM, Kabacan, North Cotabato	117	D 101 B BPI	BPI Bago Oshiro, Davao City
55	Mumali Makilala	Makilala, North Cotabato	118	Cobb 1 BPI	BPI Bago Oshiro, Davao City
56	Lacson Uno USM	USM, Kabacan, North Cotabato	119	Cobb 2 BPI	BPI Bago Oshiro, Davao City
57	Duyaya USM	USM, Kabacan, North Cotabato	120	Monthong BPI	BPI Bago Oshiro, Davao City
58	Duyaya Makilala	Makilala, North Cotabato	121	Monthong Lacson BPI	BPI Bago Oshiro, Davao City
59	Kan Yao USM	USM, Kabacan, North Cotabato	122	Alcon Fancy BPI	BPI Bago Oshiro, Davao City
60	Karn Yao BPI Balindog	2BPI Balindog, Kidapawan City	123	Umali BPI	BPI Bago Oshiro, Davao City
61	Oboza USM	USM, Kabacan, North Cotabato	124	Mdur 88 BPI	BPI Bago Oshiro, Davao City
62	GD 69USM A	USM, Kabacan, North Cotabato	125	BPI Hybrid 2	BPI Bago Oshiro, Davao City
63	GD 69 Makilala	Makilala, North Cotabato	126	Lacson Chanee BPI	BPI Bago Oshiro, Davao City
64	Atabrine/Mamer BPI Balindog	Balindog, Kidapawan City	127	Duyaya BPI	BPI Bago Oshiro, Davao City
65	Atabrine/Mamer 806 BPI Balindog	Balindog, Kidapawan City	128	Karn Yao BPI	BPI Bago Oshiro, Davao City
66	DES 218 USM	USM, Kabacan, North Cotabato	129	Oboza BPI	BPI Bago Oshiro, Davao City
67	GD 69 USM B	USM, Kabacan, North Cotabato	130	Tang Chai Churot BPI	BPI Bago Oshiro, Davao City
68	DES 1500 Balindog	Balindog, Kidapawan City	131	Durio Graveolens BPI	BPI Bago Oshiro, Davao City
69	Madonna USM	USM, Kabacan, North Cotabato	132	Duraya (Butuan) BPI	BPI Bago Oshiro, Davao City
70	Durian Bato USM	USM, Kabacan, North Cotabato	133	Mamer 806 BPI	BPI Bago Oshiro, Davao City
71	Puyat x Mdur P2 USM	USM, Kabacan, North Cotabato	134	BPI Hybrid 2 BPI	BPI Bago Oshiro, Davao City
72	Puyat x Mdur P1 USM	USM, Kabacan, North Cotabato	135	BPI Hybrid 1 BPI	BPI Bago Oshiro, Davao City
73	Puyat x D24 P22 USM	USM, Kabacan, North Cotabato	136	Palawan 1 BPI	BPI Bago Oshiro, Davao City
74	Puyat x D24 P23 USM	USM, Kabacan, North Cotabato	137	Palawan 2 BPI	BPI Bago Oshiro, Davao City
75	Kradumtong USM	USM, Kabacan, North Cotabato	138	Monthong BPI	BPI Bago Oshiro, Davao City
76	Rajah Kunit USM	USM, Kabacan, North Cotabato	139	D-24	BPI Bago Oshiro, Davao City
77	Unidentified Makilala	Makilala, North Cotabato	140	Duraya	BPI Bago Oshiro, Davao City
78	Maridagao A BPI Balindog	Balindog, Kidapawan City	141	Soriano BPI	BPI Bago Oshiro, Davao City
79	Maridagao B BPI Balindog	Balindog, Kidapawan City	142	BPI 004 CC	Cawayan, Calinan, Davao City
80	D-24 Makilala	Makilala, Kidapawan City	143	BPI 006 CC	Cawayan, Calinan, Davao City
81	Accession 2 Makilala	Makilala, North Cotabato	144	# 5 FO	Federico Ogeo, Calinan, Davao City
82	BPI 16 AC	Aroman, Carmen, North Cotabato	145	# 4 FS	Francisco Sasing, Calinan, Davao City
83	Aroman 14 AC	Aroman, Carmen, North Cotabato	146	12 FS	Francisco Sasing, Calinan, Davao City
84	BPI 013 AC	Aroman, Carmen, North Cotabato	147	21 FS	Francisco Sasing, Calinan, Davao City
85	Native 1 Kulaman	Kulaman, Sultan Kudarat	148	4 FS	Francisco Sasing, Calinan, Davao City
86	Native 2 Kulaman	Kulaman, Sultan Kudarat	149	20 FS	Francisco Sasing, Calinan, Davao City
87	Native 3 Kulaman	Kulaman, Sultan Kudarat	150	# 9 FS	Francisco Sasing, Calinan, Davao City
88	Native 7 Squibel Kulaman	Kulaman Squibel Farm, Sultan Kudarat	151	# 18 FS	Francisco Sasing, Calinan, Davao City
89	Native 6 Squibel Kulaman	Kulaman Squibel Farm, Sultan Kudarat			
90	Native 4 Squibel Kulaman	Kulaman, Sultan Kudarat			
91	Native Idio Farm KULaman	Kulaman Farm, Sultan Kudarat			

<i>Durian Cultivars /Accessions collected</i>		<i>Location</i>	<i>Primers</i>	<i>Sequence</i>	<i>Allele size range</i>	<i>No. of alleles</i>
152	8 FS	Francisco Sasing, Calinan, Davao City		3'		
153	13 FS	Francisco Sasing, Calinan, Davao City	R- 3'-ATC TAC ACC GGT GCG AAG AG-	5'		
154	11 FS	Francisco Sasing, Calinan, Davao City	9. DPL0912	F- 05'-TTC GTC GGA CTT GAG GAG AC-	501-67	135
155	10 FS	Francisco Sasing, Calinan, Davao City		3'		
156	2 KSC	Kapitana, Sirib, Calinan, Davao City		R- 3'-ACA CCA CCC AAC ACA ACT CA-		
157	14 FS	Francisco Sasing, Calinan, Davao City		5'		
158	BPI 003 CC	Cawayan, Calinan, Davao City	10.SHIN-	F- 5'-AAC GCG AAG AAG TTC TGC AT-	286-111	133
159	BPI 007 CC	Cawayan, Calinan, Davao City	1574	3'		
160	# 3 KSC	Kapitana, Sirib, Calinan, Davao City		R- 3'-TTT GCC CAT AAG CAT TGA CA-		
161	6 FS	Francisco Sasing, Calinan, Davao City		5'		
162	# 17 <sub>1</sub> FS	Francisco Sasing, Calinan, Davao City	11.SHIN-	F- 5'-ACC CAA CTC CCT CTC TTT GC-3'	490-120	139
163	# 17 <sub>2</sub> FS	Francisco Sasing, Calinan, Davao City	1621			
164	BPI 005 CC	Cawayan, Calinan, Davao City		R- 3'-TGT TGC TTG TTG GCT GTT GT-5'		
165	BPI 008 CC	Cawayan, Calinan, Davao City	12.CGR5238	F- 5'-GTG GCG TTG TTG GTG GTG-3'	501-56	103
166	# 15 FS	Francisco Sasing, Calinan, Davao City		R- 3'-TGG AAG TTC TGC AAA GCT AAT		
167	BPI 002 CC	Cawayan, Calinan, Davao City		G-5'		
168	16 FS	Francisco Sasing, Calinan, Davao City	13.DZCAG	F- 5'-CAT TGG GAG CCA GAC CAA AC-	1750-168	124
169	3 KSC	Kapitana, Sirib, Calinan, Davao City	01	3'		
170	Brunei 1 BPI	BPI Bago Oshiro, Davao City		R- 3'-CGA ACG CAT AAA ACG GAG AG-		
171	Brunei 2 BPI	BPI Bago Oshiro, Davao City		5'		
172	Brunei 3 BPI	BPI Bago Oshiro, Davao City	14.DZG 01	F- 5'-GCT TAG GCA TAC GGA GTT CA-3'	56-43	37
173	Brunei 4 BPI	BPI Bago Oshiro, Davao City		R- 3'-AGT AAG GTA CAG ATT GGG G-5'		
174	Brunei 5 BPI	BPI Bago Oshiro, Davao City	15.DZCAG	F- 5'-TGG CTT AGG CAT ACG GAG TT-3'	404-58	187
175	Lacson 1 BPI	BPI Bago Oshiro, Davao City	02			
176	Thornless Oshiro BPI	BPI Bago Oshiro, Davao City		R- 3'-AGT AAG GGT ACA GAT TCG GG-		
177	# 3 BPI BO	BPI Bago Oshiro, Davao City		5'		
178	UN 3 BPI	BPI Bago Oshiro, Davao City	16.DZTGGC	F- 5'-CGC AGA ACC TGT CGA AAA-3'	490-78	187
179	Un. BPI BO	BPI Bago Oshiro, Davao City				
180	I Brunie BP BOI	BPI Bago Oshiro, Davao City		R- 3'-CAA CCG AAT CCG TAT CCT CAA		
181	BPI 022 BK	Babsi, Kibawe, Bukidnon	17.DZA 01	F- 5'-GGC CAC AAA ATC TTC TCC AC -3'	2375-120	131
182	BPI 027 BK	Babsi, Kibawe, Bukidnon		R- 3'-CCA ACA CCA TCG TCC TAC TT-5'		
183	BPI 019 BK	Babsi, Kibawe, Bukidnon	18.CGR5130	F- 5'-GCT GAG GGA CCC TTC AAT TT-3'	2000-111	131
184	BPI 020 BK	Babsi, Kibawe, Bukidnon		R- 3'-AGT CGT AGA TGCCGG TGA AG-5'		
185	Arancillo Zam	Naga, Zamboanga del Sur	19.DZGCCG	F- 5'-GGT GGG TTC AAG CAC ATC TT-3'	1750-46	148
186	Monthong Zam	Naga, Zamboanga del Sur	01			
187	Thornless Zam	Naga, Zamboanga del Sur		R- 3'-CAT TGG GAG CCA GAC CAA AC-		
				5'		
			20.CGR5018	F- 5'-TTT GTT TGC TAG GCA TTC TC-3'	2375-179	99
				R- 3'-AAA GAG GGA GGA AAG AAG		
				CA-5'		
			21.CGR5028	F- 5'-CCT TCC CTC ATT TCC AT-3'	1750-190	89
				R- 3'-ACA TCG TTA TCC TCG AAC GG-5'		
			22.CGR5110	F- 5'-TCC TCG AGG GTT TCT TTC CT-3'	3000-105	88
				R- 3'-AAA TGC TGA AGA GAG AAG		
				CGA-5'		
			23.DZC 01	F- 5'-ATC CTG CTG CTG GTT CTG TT-3'	1750-129	112
				R- 3'-AAC TAG GAC GAT GGT GTT GG-		
				5'		
			24.CGR5100	F- 5'-TTC AGC AGT GAT TGC TTG TC-3'	3000-140	142
				R- 3'-AGG ATA TCG GCC CAT GAG A-5'		
			25.CGR5117	F- 5'-GGT TCG CTG GGA GAT GAT AA-3'	2000-111	132
				R- 3'-CAG GCT GTT TCC TCC AGC TA-5'		
			26.CGR5136	F- 5'-CTT TCC CAC CCA TTC CAA A-3'	1437-62	122
				R- 3'-CGT TGC CGT CCT CTG ATA AT-5'		
			27.CGR5141	F- 5'-TAC GGA ATC GGA GAG TCG AG-	2687-264	118
				3'		
				R- 3'-TTA GTC CAC CAA GAG CAG GG-		
				5'		
			28.CGR5030	F- 5'-ATT AAG CCC AAT GCT GAT GG-3'	3000-367	162
				R- 3'-AGT CGA AGA TGT TGC TGC TGC		
				TG-5'		
			29.CGR5091	F- 5'-CAT GGC CAC TAC TGG AAA CA-3'	3000-42	101
				R- 3'-ACA ATT CAT CCA GAC CCA GG		

TABLE II  
LIST OF INFORMATIVE SSR PRIMERS SCREENED FOR THEIR UTILITY IN  
IDENTIFYING DURIAN COLLECTIONS

<i>Primers</i>	<i>Sequence</i>	<i>Allele size range</i>	<i>No. of alleles</i>
1. CGR5334	F- 5'-AGC ATT GAG GGC CTT TGT TT-3' R- 3'-ACT TGC CAC GTT CAT CAC AA- 5'	1125-50	128
2. CGR6723	F- 5'-CTA GGT CGA TGC TCT CTG GC-3' R 3'-CCG ATC TAT CCG AGA AGC TG-5'	404-37	130
3. CGR6729	F- 5'-AGT GCC AGA GAT ACG GGA AA- 3' R- 3'-TGC AGG AGA ATG GAA GCT CT- 5'	501-168	109
4. CGR6987	F- 5'-CTG GTC TGC TTC TCC TCA CC-3' R- 3'-CCT TGC TGA CCA AGA AGA GC- 5'	2062-78	97
5. DPLO056 4	F- 5'-GTT AAT GCT CTC CCT CCC TCT C-3' R- 3'-TAA GGC TAA GAG GCC TGC AA- 5'	490-124	155
6. DPLO072 5	F- 5'-CTG TCA CCA TCG TTG ACC AC-3' R- 3'-TAC ATT CAT TCG GTG ATG GCT- 5'	490-130	146
7. DPLO079 0	F- 5'-ACA ATG GCG GAT TGG ATT C-3' R- 3'-TTC CAA GTG TCA CCC TCT CAC- 5'	480-81	136
8. DPL0911	F- 5'-ACA GGT GAC GAT TCC TGG AG-	480-85	123

*A. Development and Screening of Molecular Markers (SSR) Protocol for Durian*

One hundred twenty seven (127) SSR primers were evaluated and screened to determine their utility in discriminating the 187 clones of Durian samples collected. Out of these 127 primers, 29 were found informative. Moreover, from these 29 SSR primers, 26 (Table II) were found useful in distinguishing the samples as evidenced by their high discrimination power (PD) in generating the unique alleles (Tables III & IV). Notably, primer CGR6987 gave the highest discriminating power (PD=0.98). This result showed that SSR primers can be used to efficiently determine genetic similarities of Durian clones, collected. However, Two (2) of these primers, DZG01 (PD=0.64) and CGR 5238 (PD=0.70) although with high PD were not able to determine unique alleles from the 187 Durian clones.

TABLE III  
CHARACTERISTICS OF 29 MICROSATELLITES WHICH AMPLIFIED SUBSTANTIAL PCR PRODUCT WITH 187 DURIAN ACCESSIONS

Primers	No. of Genotypes Generated	No. of heterozygous genotypes	H <sub>a</sub>	H <sub>E</sub>	PD
CGR5130	131	66	0.50	0.84	0.96
CGR5334	128	37	0.29	0.78	0.90
CGR6723	130	9	0.07	0.36	0.56
DZG01	37	3	0.08	0.50	0.64
CGR5018	99	30	0.30	0.77	0.91
CGR5028	89	22	0.25	0.75	0.87
CGR6987	97	69	0.71	0.89	0.98
CGR5110	88	28	0.32	0.81	0.85
DZC01	112	43	0.38	0.80	0.94
CGR5100	142	38	0.27	0.81	0.93
DZA01	131	20	0.15	0.80	0.83
CGR5238	103	24	0.23	0.50	0.70
DZCAG01	124	19	0.15	0.84	0.86
CGR6729	109	40	0.37	0.83	0.95
CGR5117	132	48	0.36	0.82	0.94
CGR5136	122	26	0.21	0.81	0.89
CGR5141	118	34	0.29	0.84	0.89
CGR5030	162	59	0.36	0.88	0.93
CGR5091	101	42	0.42	0.83	0.92
DZTGCC01	187	2	0.01	0.34	0.93
DZGCC01	148	21	0.14	0.15	0.89
DPL0725	146	40	0.27	0.71	0.93
DPL0790	136	49	0.36	0.73	0.96
DZCAG02	187	3	0.02	0.36	0.11
SHIN1621	139	58	0.42	0.38	0.95
DPL0912	135	67	0.50	0.81	0.97
DPL0564	155	51	0.33	0.81	0.96
DPL0911	123	25	0.20	0.68	0.85
SHIN1574	133	33	0.25	0.81	0.81
<b>Total</b>	<b>3644</b>	<b>1006</b>			

TABLE IV  
UNIQUE ALLELES/GENOTYPES GENERATED BY 29 SSR MARKERS FROM EACH OF THE 187 DURIAN CLONES COLLECTED

Loci	No. of Unique Alleles	Genotypes	Clones
1. CGR5130	48	AC	Native3Tamp
		ACIJK	NativBanga
		AF	ArancilloB.Balindog
		ADFIJ	Puyat1BPI
		AJ	PuyatUSM
		ACDIJ	Cobb1USM
		ACDFIJKN	MonthongBPI
		ACDEFGIJK	AlconFancyUSM
		ACDGIJK	AlconFancyMak
		AIJK	UmaliBPI
		ABDFIJK	MDur88BPI
		ABCDFIJ	MDur88USM
		ABCDGFIJKN	Hybrid2BPI
		AGIN	UmaliMonthUSM
		AFIJK	KarnYaoBPI.Bal
		DD	ObozaUSM
		ABM	SuspMonthPol
		ABDFHIJMN	DES218USM
		ABDFHIM	DES1500.Bal
		ABDFH	DurianBatoUSM
		ABDFMN	PuyatxMdurP1USM
		ABDFGM	PuyatxD24P22USM
		AHM	SolomonPol
		ABDHJMN	UnID.2Line1Pol
		AMN	D-24TSF
		AHM	L2T1Pol
		ABDFGHIJKL	TSF2
		MM	UnID.1Kul
		ABDFH	UnID.2Kul
		ABDEHIJM	UnID.4Kul
		ABDEHIJKM	4FS
		ABDI	BPI004CC
		ABHIMN	BPI006CC
		ABDHIJM	#4FS
		ABDHIJMN	12FS
		ABHM	BPI16AC
		ABDIMN	Aroman14AC
		ABDIM	13FS
		ABDEHKM	2KSC
		ABD	BPI003CC
		MN	BPI007CC
		ABDMN	BPI027BK
		ABDHI	BPI019BK
		ABDN	BPI020BK
		ABDH	BPI008CC
		ABEHIKL	Brunei1.BPIbago
		ABDHILM	Brunei2.BPIbago
		ABHILM	Brunei5.BPIbago
2. CGR5334	18	ACG	NativTSFbark
		BCEFG	Puyat1BPI
		BC	Puyat2BPI
		BCDF	D.101.B.BPI
		BDFG	D101.USM
		BCFG	Cobb1BPI
		BG	Cobb2.BPI
		BCDEFG	Cobb1USM
		ACDFG	MDur88USM
		ABCDG	DuyayaBPI
		AG	KarnYaoBPI
		FI	ObozaBPI
		FGI	ObozaUSM
		CFH	PuyatxMdurP2USM
		ABCD	TSF2
		ABCDF	UnID.4Kul

Loci	No. of Unique Alleles	Genotypes	Clones	Loci	No. of Unique Alleles	Genotypes	Clones
3. CGR6723	3	CGH	BPI008CC	7. CGR5110	16	CD	MaridagaoBpiBal
		GH	BPI013AC			ABCDJ	UnID.1Pol
		BB	NativeBanga			BCDJ	ChaneeIdioKul
4. CGR5018	14	ABD	ArancilloUSM	8. DZC01	19	BCFG	BPI16AC
		ABC	PuyatUSM			JL	I.BrunieBPIbago
		ABCDFI	Puyat1BPI			GJ	#3KSC
		AEI	D101.USM			GIJKLM	#17 <sub>1</sub> FS
		II	Mdur88BPI			DFGHIJKN	#17 <sub>2</sub> FS
		CDI	Mdur88USM			DJK	BPI005CC
		ADI	DuyayaBPI			JK	BPI020BK
		EI	Pal1.BPIbago			FJK	BPI008CC
		EE	MonthBPIbago			EFGHIJKMN	BPI002CC
		ACDEFGHI	KradumtongUSM			FGHIJKM	Brunei1.BPIbago
		ACDEFHI	RajahKunyitUSM			FGHIJKM	Brunei4.BPIbago
		ACDEFI	ChaneeIdioKul			FGJKM	Brunei5.BPIbago
		ACDEGI	UnID.3BPIbago			AJL	NativePol
		ACG	4FS			AJ	Nativ10IdioKul
ACDEG	20FS	AH	ChanIdioKul				
5. CGR5028	7	ABCG	BPI006CC	9. CGR5100	18	ACFGHIJKL	ArancilloB.Balindog
		ABCDFG	ObozaUSM			ABCDL	Cobb1USM
		ABCDEG	RajahKunyitUSM			ABCDEFGHIJL	MonthongBPI
		ABCD	ArancilloZam			ABCEFGHIL	MonthongUSM
		ABDEG	Aroman14AC			ABCFGHIJL	AlconFancyUSM
		ABCDE	Brunei1.BPIbago			ABCDFGHIJKL	AlconFancyMak
		ABC	Brunei2.BPIbago			ABCDGHIJKL	UmaliBPI
6. CGR6987	56	ABG	Brunei4.BPIbago	9. CGR5100	18	AB	UnIDPol
		BCEFGHMN	NativeTSFdup			ABCDGHL	MDur88BPI
		BCHMN	Native7SquibelKul			ABCDEFGHL	Hybrid2BPI
		BC	Nativ6SquibelKul			HKL	DuyayaUSM
		AB	Nativ11IdioKul			ABCDHKL	KarnYaoBPI.Bal
		CDEFGIKLMN	Nativ12IdioKul			HK	Duraya(But)BPIBago
		BL	ArancilloUSM			ABEFGHK	GD69USM
		BCDEFGIKLMN	ArancilloC.Balindog			AHK	MaridagaoBpiBal
		BCDEFGIM	ArancilloKul			CEFH	NativePol
		ABCGI	ChaneeBPI			BDEFH	NativBanga
		ABCEFGHILMN	ChaneePol			ADEFH	NativIdioFarmKul
		ABCDEFGHILM	ChaneeMasilimKul			ABEFH	NativMasilimKul
		ABGI	ChaneeKul			DE	Chanee2815BPI
		CEGIL	Puyat1BPI			EE	ChaneePol
		ABCDEGJ	D101.B.BPI			CDEF	Puyat2BPI
		ABDGJ	D101.USM			CDEH	AlconFancyUSM
		BCDEM	Cobb1USM			BCDE	DuyayaBPI
		EFJLM	MonthongBPI			BCDEFH	KarnYaoBPI
		BDEFMLN	MonthongUSM			AA	MadonnaUSM
		ABCFJM	MonthongLacsonBPI			ABE	ChaneeIdioKul
		BCFJM	AlconFancyBPI			ABCDFGH	ArancilloZam
		BCFJKLMN	AlconFancyUSM			CE	#3KSC
		BCEFHIJKLMN	AlconFancyMak			BCEF	16FS
		BCJN	UmaliBPI			CEF	3KSC
		BCDFJKL	MDur88BPI			EFGH	Brunei1.BPIbago
		CDEFIJKLMN	Mdur88USM			ACEFH	Brunei5.BPIbago
		CJM	UmaliMonthUSM			ABDEFH	Lacson1.BPIbago
		BJM	LacsonChaneeBPI			ABCDF	ChaneeUSM
		AJ	ChaneeYolF.Tupi			ABDFGKL	ChaneePol
		BCEFIJKM	DuyayaBPI			ADFG	Puyat2BPI
		BCEFIJKLM	DuyayaUSM			DEGKL	D101.B.BPI
		BCDEFJ	KarnYaoBPI			ABDGKL	Cobb1BPI
		BCDEJLMN	KanYaoUSM			ACDEFGKL	Cobb1USM
		JMN	KarnYaoBPI.Bal			DEFGKL	ObozaBPI
BCDELN	ObozaBPI	DFG	SuspMonthPol				
BJLN	SuspNanam4Pol	DGK	RedPrunTSF				
AD	At/mamBPIBal.A	ADF	BPI006CC				
CDJ	GD69USM	DEF	#4FS				
CM	MadonnaUSM	ABCDEFKL	14FS				
BCFJ	DurianBatoUSM	BCDFKL	#3KSC				
ABCDGHMN	RajahKunyitUSM	ABCDEFGKL	BPI027BK				

Loci	No. of Unique Alleles	Genotypes	Clones	Loci	No. of Unique Alleles	Genotypes	Clones
10. DZA01	7	ADFKL	#15FS	15. CGR5136	10	ABCDEGH	At/mamBPIBa.A
		DFGHKL	3KSC			AH	Hyb1BPIbago
		ADEFIJKL	Brunei2.BPIbago			EG	UnID.2Line1Pol
		DEKL	Brunei4.BPIbago			EE	RedPrunTSF
		ABDEF	NativS.milagrosaTamp			BCDEGHIK	TSF2
		BCFG	D101.USM			BCGH	ThornlessZam
		CC	CobbBanga			CDGH	12FS
11. CGR5238	16	FG	DuyayaUSM	16. CGR5141	20	ABCDGHL	8FS
		BB	2KSC			ABCDEGIK	BPI019BK
		BCDEF	14FS			ABG	BPI005CC
		ABCFG	Lacson1.BPIbago			BB	16FS
		ADE	Native7SquibelKul			BGH	BPI013AC
		ADEHI	ArancilloUSM			ABCDEGI	Brunei4.BPIbago
		ABCDEFGHI	Puyat1BPI			ABCDEGHI	Brunei5.BPIbago
		GHI	Puyat2BPI			ABCDHI	ThornlessBPIbago
		AHI	Cobb1BPI			ABGHI	NativIdioFarmKul
		ADEGH	MonthongUSM			GI	ChanIdioKul
		ABCDFH	MDur88USM			ABDEG	ArancilloBPI
		GH	DuyayaBPI			ADGI	ArancilloBanga
		ABCDEFGH	SuspNanam4Pol			ABCDGHI	DuyayaUSM
		DH	Hyb2BPIbago			ABDHI	KarnYaoBPI
		BCDH	DES218USM			EG	SuspNanam3.Pol
		CC	RajahKunyitUSM			AG	PuyatxMdurP2USM
12. DZCAG01	9	BCH	ChaneeIdioKul	17. CGR5030	36	DD	MonthongZam
		ACH	ArancilloZam			ADEFGHI	Brunei1.BPIbago
		ABDEFH	Brunei1.BPIbago			BCE	NativePol
		ABDEH	Brunei2.BPIbago			BE	Native7SquibelKul
		ABCDEFGH	ArancilloUSM			DEF	NativBanga
		CDEFG	MonthongLacsonBPI			FH	ArancilloKul
		ABCFG	DuriogravBPI			EG	ChaneeUSM
		CC	Mamer806.BPIbago			BDF	Puyat1BPI
		ABCDEG	DES218USM			CDEFHG	D101.B.BPI
13. CGR6729	22	ABCEFG	BPI16AC	14. CGR5117	23	CC	MonthongTSF
		ABEFG	#9FS			CDEFG	LacsonChaneeBPI
		ABE	8FS			ABCDEG	SuspNanam4Pol
		AD	ThornlessBPIbago			BCEFGH	DvoSelMasilimKul
		BC	ChanIdioKul			EE	ThornlessZam
		DD	Chanee2815BPI			BCFGH	BPI022BK
		ABDEH	ChaneeBPI			BCDF	#3BPIbago
		HH	MonthongUSM			BCEF	UnID.BPIbago
		II	CobbMak			ABCEF	BPI16AC
		HI	MonthongBPI			BCF	13FS
		ABCDEFG	MonthongLacsonBPI			BCDEF	#3KSC
		CEH	AlconFancyBPI			ACDEFGH	#15FS
		BCEH	AlconFancyUSM			BCDG	Brunei1.BPIbago
		ABCEH	AlconFancyMak			ADEGHI	NativS.milagrosaTamp
		ABDEFGHI	MDur88USM			AE	NativeKul
		ABDEGHI	UmaliMonthUSM			AD	Native2Kul
		ACDEFGHI	DuyayaUSM			ACEFGHI	NativeTamp
		CD	At/mamBPIBa.A			ACDEFGHIL	Native7SquibelKul
		ABDEF	SorianoBPIbago			ADEFGHIKL	ArancilloUSM
		ACDEFGH	4FS			HIKL	ArancilloBPI
		ABCEGHI	#17 <sub>2</sub> FS			AFGH	ChaneePol
		ACEH	#15FS			ABGH	ChaneeMasilimKul
ABCDEGH	16FS	ABDEFGHIK	Puyat1BPI				
BCDEH	Brunei1.BPIbago	ADEFGHIK	Puyat2BPI				
BCDE	Brunei4.BPIbago	AIEFGHIK	PuyatUSM				
CDEH	Lacson1.BPIbago	AEFH	PuyatKul				
BCDEG	NativS.milagrosaTamp	AEFGH	PuyatTSF				
CDEG	NativePol	GL	MonthongKul				
CC	Native2Kul	EFGHL	MonthongLacsonBPI				
BC	CobbBanga	ACDEFGH	UmaliBPI				
BCDEFGHI	MonthongUSM	ACDEFH	UmaliMahF.Kul				
ABCEFGH	MDur88USM	ACDEFGHIK	LacsonChaneeBPI				
FG	UmaliMonthUSM	ABDEFGHIKL	ObozaBPI				
CEGH	LacsonChaneeBPI	HIL	ObozaUSM				

Loci	No. of Unique Alleles	Genotypes	Clones	Loci	No. of Unique Alleles	Genotypes	Clones
18. CGR5091	24	DHI	GD69.USM	23. DPL0912	45	CDFIJK	Puyat1BPI
		AEFHI	SuspNanamPol			CEIJ	PuyatTSF
		AFI	At/mamBPIBal.A			BDEIJ	D101.B.BPI
		HIK	SuspMonthPol			BCDEIJ	D.101.B.BPI
		ACDEF	Pal2.BPIbago			EIJ	CobbMak
		ADEFI	MonthBPIbago			ADEGHIJK	AlconFancyUSM
		AEFIK	DES218USM			DEGIJ	MDur88BPI
		ACDEHI	UnID.1Pol			ADEFGHIJK	UmaliMonthUSM
		AHI	UnID.2Kul			EHIJK	LacsonChaneeBPI
		ADHI	UnID.3Kul			EIJK	LacsonUnoUSM
		CEF	MonthongZam			DEFHIJK	DuyayaUSM
		ABCDEFGHJK	#5FO			ADFHIJK	KarnYaoBPI
		ACEF	BPI007CC			ADEFHIJK	GD69.USM
		ACEFI	#3KSC			AEJ	Mamer806.BPIbago
		ACDEH	3KSC			EJ	At/mam806Bal.B
		AC	NativeTamp			AIEFGJ	Hyb2BPIbago
		ABCEHI	ChaneeUSM			BJ	Pal1.BPIbago
		FHI	Cobb2.BPI			AIEFGJ	DES218USM
		EFHI	Cobb1USM			AIJ	PuyatxD24P22USM
		EHI	MonthongBPI			ADEIJK	RajahKunyitUSM
		ABCFHI	AlconFancyUSM			ADIJ	MaridagaoBpiBal
		AHI	AlconFancyMak			KK	UnID.TSF
		CC	ChaneeYoIF.Tupi			ADEGIJ	ArancilloZam
		ACDHI	KarnYaoBPI.Bal			ABCDEFIJK	BPI006CC
		ACDFHI	GD69.Mak			ABDEFGIJK	21FS
		AA	SuspNanam3.Pol			ABCDEFGIJK	#3BPIbago
		FF	MonthBPIbago			AIEFGHIJK	UnID.BPIbago
		ABCHI	PuyatxMdurP2USM			ABCDFGHIJK	BPI16AC
		BCDEHI	KradumtongUSM			HIJK	6FS
		BDHI	RajahKunyitUSM			ACEFIJK	Brunei3.BPIbago
		ABCG	BPI022BK			AFIJK	Brunei4.BPIbago
		BCJ	BPI006CC			AEFIJK	Lacson1.BPIbago
		ACIJ	12FS			AEGIJK	ThornlessBPIbago
BC	#3BPIbago	BIJ	NativS.milagrosaTamp				
BIJ	8FS	BG	Native2Kul				
ABCFIJ	10FS	ACEI	Nativ4SquibelKul				
ABCGIJ	#3KSC	BDFGHIJ	Nativ10IdioKul				
ABCHIJ	#17 <sub>2</sub> FS	BCD	Nativ12IdioKul				
BCEHIJ	Brunei1.BPIbago	BC	ArancilloUSM				
19. DZGCC01	1	ABCDEF	#17 <sub>2</sub> FS	BB	ArancilloC.Balindog		
20. DPL0725	2	BDHI	Nativ9IdioKul	CDEGH	ArancilloKul		
21. DPL0790	19	ABI	Nativ11IdioKul	BF	Chanee2815BPI		
		FF	NativeTamp	HI	ChaneePol		
		ABCDH	ChanIdioKul	IJ	ChaneeMasilimKul		
		BDF	ArancilloBanga	ABCDEGHIJ	ChaneeKul		
		CI	ArancilloKul	ABCEHI	Puyat2BPI		
		DGH	PuyatUSM	DHI	Puyat2Mak		
		ABCFHI	PuyatMak	ABCEI	PuyatTSF		
		CDFG	Cobb1USM	BCIK	Cobb1USM		
		AA	CobbBanga	BCDGIH	MonthongBPI		
		CDG	MonthongUSM	BCGHI	AlconFancyMak		
		BCFGH	MDur88BPI	BHI	MDur88BPI		
		ABCDEFH	Mdur88USM	BCFGHI	MdurKul		
		ACDFGH	KanYaoUSM	GI	UmaliMahF.Kul		
		CDH	SuspNanam1.Pol	BCEG	LacsonUnoUSM		
		ACDF	MadonnaUSM	BCDEGHIK	DuyayaUSM		
		BCDEF	RajahKunyitUSM	ABCDHI	SuspNanamPol		
		ABCDF	MaridagaoBpiBal	ACDI	SuspNanam4Pol		
		BCEFG	UnID.3BPIbago	AB	DuriogravBPI		
		BDG	BPI16AC	ABCHI	Pal1.BPIbago		
		ABCD	BPI013AC	BCGJ	DurianBatoUSM		
		22. SHIN1621	37	ADIJK	Native2Kul	BCFGHJ	PuyatxMdurP2USM
ACIJK	Nativ4SquibelKul	ABCDEFGH	PuyatxD24P22USM	ABCDEFGHIJ	KradumtongUSM		
BDFGIJK	Nativ12IdioKul	ABFGHI	SolomonPol	ABCDE	TSF2		
BCDFGIJK	ArancilloUSM						
CDFIJ	ChaneeKul						



Loci	No. of Unique Alleles	Genotypes	Clones
24. DPL0564	24	ABGHK	UnID.1Kul
		AK	UnID.3Kul
		ABDEFGHK	UnID.4Kul
		ABCDEFGHK	MonthongZam
		ABCEFGHK	4FS
		ABCDEHK	20FS
		BCHJ	BPI022BK
		BCEHJ	#5FO
		BCEGHJ	#4FS
		BEFGHJK	8FS
		BCEFGHJK	13FS
		BCFH	ThornlessBPIbago
		ACDH	NativS.milagrosaTamp
		CDH	NativeTamp
		ABCDFGH	Native7SquibelKul
		ACDFH	Nativ6SquibelKul
		AFGH	NativBanga
		ABCDEFH	ChanIdioKul
		ADEGH	Nativ11IdioKul
		BDEFH	NativMasilimKul
		DFGH	ArancilloBPI
		CHI	Chanee2815BPI
		DEFHI	PuyatUSM
		II	PuyatIdioKul
BCDFGHI	PuyatTSF		
CFHI	AlconFancyUSM		
ABCDFGHI	UmaliMonthUSM		
ACDFGHI	KamYaoBPI.Bal		
GHI	NativPol		
DF	DuriogravBPI		
ABD	Hyb2BPIbago		
ABCDEFHI	MaridagaoBpiBal		
BHI	RedPrunTSF		
FF	MonthongZam		
ABDHI	I.BrunieBPIbago		
ABCDFHI	3KSC		
25. DPL0911	11	ADEF	ArancilloC.Balindog
		ACE	Chanee2815BPI
		CD	ChaneePol
		ABCG	PuyatUSM
		ACF	Mamer806.BPIbago
		ABDE	Pal2.BPIbago
		ABCDF	MaridagaoBpiBal
		AG	4FS
		ABCE	BPI022BK
		ABD	#3BPIbago
		ABCDG	BPI008CC
26. SHIN1574	18	ABDGH	NativIdioFarmKul
		BB	Nativ10IdioKul
		ACDE	ChanIdioKul
		ABCDE	ArancilloB.Balindog
		ACFGH	ChaneeBPI
		ABCDEGH	MonthongLacsonBPI
		ACDFG	TangChaiChurotBPI
		GG	Duraya(But)BPIBago
		ABCDFG	Pal2.BPIbago
		ABCDGH	PuyatxD24P23USM
		CC	UnID.3Kul
		ABC	UnID.4Kul
		ADFGH	BPI022BK
		ADGH	BPI006CC
		AD	#3BPIbago
		ACDF	UnID.BPIbago
		ADEFG	BPI16AC
		AGH	BPI003CC

### B. Microsatellite Polymorphism

From the 29 primers selected, distinguishable alleles ranging from 2-15 were detected in the clones evaluated per primer. This revealed polymorphism at individual loci (Table III). Overall, the said 29 primers detected a total of 3644 genotypes from the 187 clones evaluated. Specifically, primer CGR 6987 detected fifteen (15) alleles from the 187 Durian samples evaluated; followed by primer CGR 5130 which identified fourteen (14) alleles. On the other hand, primer DZG01 was able to detect only two (2) distinguishable alleles. Table IV also shows the primers that generated unique genotypes. Different genotypes were produced based on the unique alleles detected by the said informative SSR primers selected.

Primers CGR 6987 identified 56 clones with unique genotypes; primer CGR 5130 had detected 48 clones with unique genotypes; primer DPL 0912 detected 45 clones with unique genotypes; primer SHIN 1621 detected 37 clones with unique genotypes; primer CGR 5030 identified 36 clones with unique genotypes; primer CGR5091 identified 24 clones with unique genotypes; Primer CGR 5117 detected 23 clones with unique genotypes; primer CGR 6729 detected 22 clones with unique genotypes; primer CGR5141 identified 20 clones with unique genotypes; primers DZC01 and DPL 0790 detected 19 clones with unique genotypes; primers CGR 5334 and SHIN 1574 identified 18 clones with unique genotypes; primers CGR 5110 and CGR 5238 detected 16 clones with unique genotypes. While primer CGR 5018 identified 14 clones with unique alleles. Moreover, primer DPL 0911 detected 11 clones with unique genotypes; primer CGR 5136 detected 10 clones with unique genotypes; primer DZCAG01 detected 9 unique clones; primer DZA01 and CGR 5028 detected 7 unique alleles; CGR 6723 identified 3 unique alleles; primer DPL 0725 detected 2 clones with unique alleles. Primer DZGCC01 detected 1 clone each with different genotype unique for the specific clone. A detailed DNA profile of the different clones detected by the 29 primers had been generated. It is interesting to note that DZGCC01 generated unique and specific alleles for a clone collected from Francisco Sasing, Calinan, Davao City DPL 0725 on the other hand is unique and specific for a native variety collected from Kulaman, Sultan Kudarat. These primers therefore, can be used as markers for authenticating and identifying the said clones. Thus, the results obtained can assist BPI in their routine testing for the certification purpose.

Fig. 1 shows a sample of the genetic profile of the different cultivars as detected by the primer DPL0912. A closer inspection of the figure shows that those bands represented by the same color have the same genetic profile while those represented by different colors are different from each other hence they can be used to distinguish one clone over the other clones.

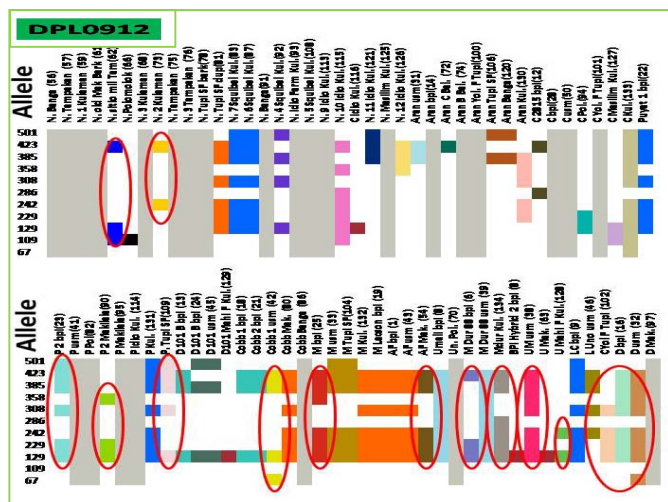


Fig. 1 Sample of DNA profile of the durian cultivars detected by SSR primer DPL0912

Fig. 2 on the other hand shows the genetic similarities of the 187 clones based on the 29 SSR primers. Cluster analysis revealed that at 0.64 similarity coefficient, the 187 clones can be grouped into 128 clusters. Furthermore, the dendrogram shows that clone BPI027 from Babsi, Kibawe, Bukidnon and clone 6FS from Francisco, Sasing, Calinan, Davao del Sur are the closest relatives while the most distant clones are #3 BPI Bago Oshiro from Bago Oshiro, Davao City and Cobb 1 from USM. The dendrogram also shows that even if 2 cultivars have the same name, they are genetically different. For example Arancillo 2 from Balindog is genetically different from Arancillo of BPI Davao or Arancillo of Banga, South Cotabato. This implies therefore, that these cultivars with same names are either mislabeled or of different strains.

The overall results showed that the variability found among the clones using the microsatellites was high enough to distinguish the clones used in this project. Primers can be selected and used as markers for identifying the clones of interest. With the foregoing, the project was able to develop a molecular marker technique that can guide breeders in future improvement work. This will also fast track and accurately authenticate questionable materials during certification undertakings of the Bureau of Plant Industry. It is also noteworthy that this study is a pioneering work in the Philippines and even in Southeast Asia. So far, this is the first venture in authentication and identification of the said clones. From this, we hope to develop a dip stick assay that can be done in the field without necessarily using costly and specialized or sophisticated equipment. The USM Genetic and Molecular Biology laboratory can now assist the Bureau of Plant Industry in their Durian certification activities.

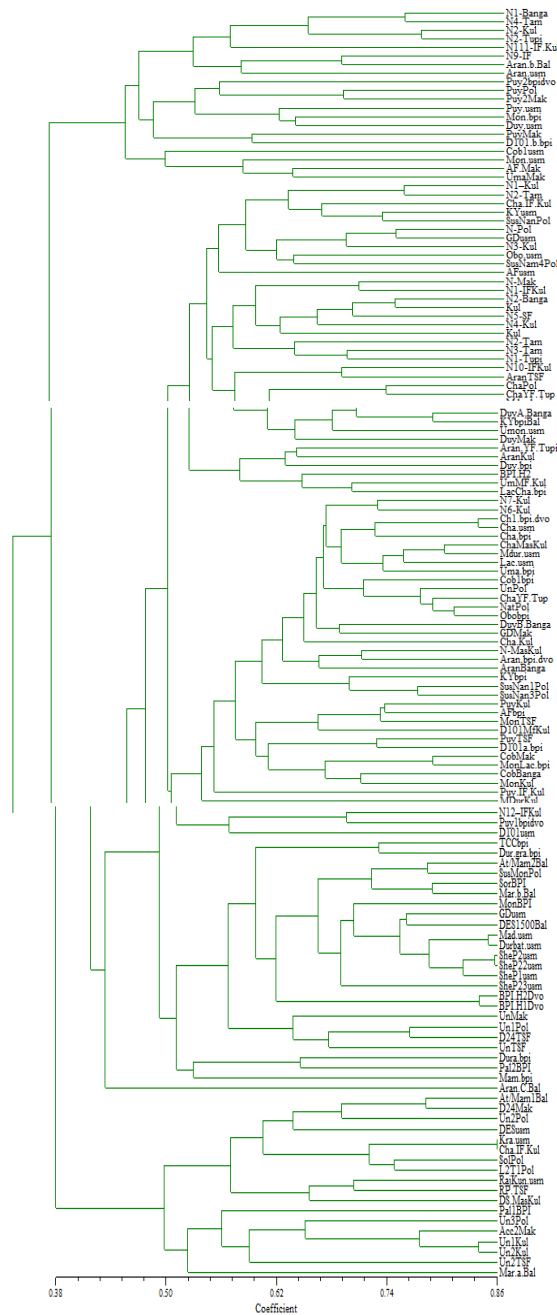


Fig. 2 Dendrogram of 187 Durian clones constructed by NTSYSpc version 2.10z and clustered by UPGMA [4] based on 21 SSR markers

#### IV. SUMMARY AND CONCLUSION

Our results demonstrate the relevance of using molecular techniques in evaluating and identifying durian clones. The most polymorphic primers tested in this study could be useful tools for detecting variation even at the early stage of the plant especially for commercial purposes. The process developed combines the efficiency of the microsatellites development process with the optimization of non-radioactive detection process resulting in a user-friendly protocol that can be performed in two (2) weeks and easily incorporated into

laboratories about to start microsatellite development projects. This can be of great importance to extend microsatellite analyses to other crop species where minimal genetic information is currently available. With this, the University can now be a service laboratory for routine testing and authentication of durian clones.

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