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

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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

DURIAN, (*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

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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 dendogram 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:

$$\text{PIC} = 1 \sum_{\text{i=1n}} Pi^2$$

where: pi = frequency of the ith 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

	Durian Cultivars /Accessions collected	*Location*
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 Farn	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 Farn 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

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

	Durian Cultivars /Accessions collected	*Location*
152	8 FS	Francisco Sasing, Calinan, Davao City
153	13 FS	Francisco Sasing, Calinan, Davao City
154	11 FS	Francisco Sasing, Calinan, Davao City
155	10 FS	Francisco Sasing, Calinan, Davao City
156	2 KSC	Kapitana, Sirib, Calinan, Davao City
157	14 FS	Francisco Sasing, Calinan, Davao City
158	BPI 003 CC	Cawayan, Calinan, Davao City
159	BPI 007 CC	Cawayan, Calinan, Davao City
160	# 3 KSC	Kapitana, Sirib, Calinan, Davao City
161	6 FS	Francisco Sasing, Calinan, Davao City
162	# 17_1 FS	Francisco Sasing, Calinan, Davao City
163	# 17_2 FS	Francisco Sasing, Calinan, Davao City
164	BPI 005 CC	Cawayan, Calinan, Davao City
165	BPI 008 CC	Cawayan, Calinan, Davao City
166	# 15 FS	Francisco Sasing, Calinan, Davao City
167	BPI 002 CC	Cawayan, Calinan, Davao City
168	16 FS	Francisco Sasing, Calinan, Davao City
169	3 KSC	Kapitana, Sirib, Calinan, Davao City
170	Brunei 1 BPI	BPI Bago Oshiro, Davao City
171	Brunei 2 BPI	BPI Bago Oshiro, Davao City
172	Brunei 3 BPI	BPI Bago Oshiro, Davao City
173	Brunei 4 BPI	BPI Bago Oshiro, Davao City
174	Brunei 5 BPI	BPI Bago Oshiro, Davao City
175	Lacson 1 BPI	BPI Bago Oshiro, Davao City
176	Thornless Oshiro BPI	BPI Bago Oshiro, Davao City
177	# 3 BPI BO	BPI Bago Oshiro, Davao City
178	UN 3 BPI	BPI Bago Oshiro, Davao City
179	Un. BPI BO	BPI Bago Oshiro, Davao City
180	I Brunie BP BOI	BPI Bago Oshiro, Davao City
181	BPI 022 BK	Babsi, Kibawe, Bukidnon
182	BPI 027 BK	Babsi, Kibawe, Bukidnon
183	BPI 019 BK	Babsi, Kibawe, Bukidnon
184	BPI 020 BK	Babsi, Kibawe, Bukidnon
185	Arancillo Zam	Naga, Zamboanga del Sur
186	Monthong Zam	Naga, Zamboanga del Sur
187	Thornless Zam	Naga, Zamboanga del Sur

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

Primers	Sequence	Allele size range	No. of alleles
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. DPLO0564	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. DPLO0725	F- 5'-CTG TCA CCA TCG TTG ACC AC-3' R- 3'-TAC ATT CAT TCG GTG ATG GCT-5'	490-130	146
7. DPLO0790	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-3' R- 3'-ATC TAC ACC GGT GCG AAG AG-5'	480-85	123
9. DPL0912	F- 05'-TTC GTC GGA CTT GAG GAG AC-3' R- 3'-ACA CCA CCC AAC ACA ACT CA-5'	501-67	135
10.SHIN-1574	F- 5'-AAC GCG AAG AAG TTC TGC AT-3' R- 3'-TTT GCC CAT AAG CAT TGA CA-5'	286-111	133
11.SHIN-1621	F- 5'-ACC CAA CTC CCT CTC TTT GC-3' R- 3'-TGT TGC TTG TTG GCT GTT GT-5'	490-120	139
12.CGR5238	F- 5'-GTG GCG TTG TTG GTG GTG-3' R- 3'-TGG AAG TTC TGC AAA GCT AAT G-5'	501-56	103
13.DZCAG01	F- 5'-CAT TGG GAG CCA GAC CAA AC-3' R- 3'-CGA ACG CAT AAA ACG GAG AG-5'	1750-168	124
14.DZG 01	F- 5'-GCT TAG GCA TAC GGA GTT CA-3' R- 3'-AGT AAG GTA CAG ATT GGG G-5'	56-43	37
15.DZCAG02	F- 5'-TGG CTT AGG CAT ACG GAG TT-3' R- 3'-AGT AAG GGT ACA GAT TCG GG-5'	404-58	187
16.DZTGGC01	F- 5'-CGC AGA ACC TGT CGA AAA-3' R- 3'-CAA CCG AAT CCG TAT CCT CAA G-5'	490-78	187
17.DZA 01	F- 5'-GGC CAC AAA ATC TTC TCC AC -3' R- 3'-CCA ACA CCA TCG TCC TAC TT-5'	2375-120	131
18.CGR5130	F- 5'-GCT GAG GGA CCC TTC AAT TT-3' R- 3'-AGT CGT AGA TGCCGG TGA AG-5'	2000-111	131
19.DZGCCG01	F- 5'-GGT GGG TTC AAG CAC ATC TT-3' R- 3'-CAT TGG GAG CCA GAC CAA AC-5'	1750-46	148
20.CGR5018	F- 5'-TTT GTT TGC TAG GCA TTC TC-3' R- 3'-AAA GAG GGA GGA AAG AAG CA-5'	2375-179	99
21.CGR5028	F- 5'-CCT TCC CTC ATT TCC AT-3' R- 3'-ACA TCG TTA TCC TCG AAC GG-5'	1750-190	89
22.CGR5110	F- 5'-TCC TCG AGG GTT TCT TTC CT-3' R- 3'-AAA TGC TGA AGA GAG AAG CGA-5'	3000-105	88
23.DZC 01	F- 5'-ATC CTG CTG CTG GTT CTG TT-3' R- 3'-AAC TAG GAC GAT GGT GTT GG-5'	1750-129	112
24.CGR5100	F- 5'-TTC AGC AGT GAT TGC TTG TC-3' R- 3'-AGG ATA TCG GCC CAT GAG A-5'	3000-140	142
25.CGR5117	F- 5'-GGT TCG CTG GGA GAT GAT AA-3' R- 3'-CAG GCT GTT TCC TCC AGC TA-5'	2000-111	132
26.CGR5136	F- 5'-CTT TCC CAC CCA TTC CAA A-3' R- 3'-CGT TGC CGT CCT CTG ATA AT-5'	1437-62	122
27.CGR5141	F- 5'-TAC GGA ATC GGA GAG TCG AG-3' R- 3'-TTA GTC CAC CAA GAG CAG GG-5'	2687-264	118
28.CGR5030	F- 5'-ATT AAG CCC AAT GCT GAT GG-3' R- 3'-AGT CGA AGA TGT TGC TGC TGC TG-5'	3000-367	162
29.CGR5091	F- 5'-CAT GGC CAC TAC TGG AAA CA-3' R- 3'-ACA ATT CAT CCA GAC CCA GG	3000-42	101

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_o	H_E	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
DZTGGC01	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
Total	**3644**	**1006**			

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
		ABCDFGIJKN	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
		ABCDFG	DuyayaBPI
		AG	KarnYaoBPI
		FI	ObozaBPI
		FGI	ObozaUSM
		CFH	PuyatxMdurP2USM
		ABCD	TSF2
		ABCDF	UnID.4Kul

Loci	No. of Unique Alleles	Genotypes	Clones
		CGH	BPI008CC
		GH	BPI013AC
3. CGR6723	3	BB	NativeBanga
		ABD	ArancilloUSM
		ABC	PuyatUSM
4. CGR5018	14	ABCDFI	Puyat1BPI
		AEI	D101.USM
		II	Mdur88BPI
		CDI	Mdur88USM
		ADI	DuyayaBPI
		EI	Pal1.BPIbago
		EE	MonthBPIbago
		ACDEFGHI	KradumtongUSM
		ACDEFHI	RajahKunyitUSM
		ACDEFI	ChaneeIdioKul
		ACDEGI	UnID.3BPIbago
		ACG	4FS
		ACDEG	20FS
		ABCG	BPI006CC
5. CGR5028	7	ABCDFG	ObozaUSM
		ABCDEG	RajahKunyitUSM
		ABCD	ArancilloZam
		ABDEG	Aroman14AC
		ABCDE	Brunei1.BPIbago
		ABC	Brunei2.BPIbago
		ABG	Brunei4.BPIbago
6. CGR6987	56	BCEFGHMN	NativeTSFdup
		BCHMN	Native7SquibelKul
		BC	Nativ6SquibelKul
		AB	Nativ11IdioKul
		CDEFGIKLMN	Nativ12IdioKul
		BL	ArancilloUSM
		BCDEFGIKLMN	ArancilloC.Balindog
		BCDEFGIM	ArancilloKul
		ABCGI	ChaneeBPI
		ABCEFGHILMN	ChaneePol
		ABCDEFGHILM	ChaneeMasilimKul
		ABGI	ChaneeKul
		CEGIL	Puyat1BPI
		ABCDEGJ	D101.B.BPI
		ABDGJ	D101.USM
		BCDEM	Cobb1USM
		EFJLM	MonthongBPI
		BDEFLMN	MonthongUSM
		ABCFJM	MonthongLacsonBPI
		BCFJM	AlconFancyBPI
		BCFJKLMN	AlconFancyUSM
		BCEFHIJKLMN	AlconFancyMak
		BCJN	UmaliBPI
		BCDFJKL	MDur88BPI
		CDEFIJKLMN	Mdur88USM
		CJM	UmaliMonthUSM
		BJM	LacsonChaneeBPI
		AJ	ChaneeYolF.Tupi
		BCEFIJKM	DuyayaBPI
		BCEFIJKLM	DuyayaUSM
		BCDEFJ	KarnYaoBPI
		BCDEJLMN	KanYaoUSM
		JMN	KarnYaoBPI.Bal
		BCDELN	ObozaBPI
		BJLN	SuspNanam4Pol
		AD	At/mamBPIBal.A
		CDJ	GD69USM
		CM	MadonnaUSM
		BCFJ	DurianBatoUSM
		ABCDGHMN	RajahKunyitUSM

Loci	No. of Unique Alleles	Genotypes	Clones
		CD	MaridagaoBpiBal
		ABCDJ	UnID.1Pol
		BCDJ	ChaneeIdioKul
		BCFG	BPI16AC
		JL	I.BrunieBPIbago
		GJ	#3KSC
		GIJKLM	#17_1FS
		DFGHIJKN	#17_2FS
		DJK	BPI005CC
		JK	BPI020BK
		FJK	BPI008CC
		EFGHJKMN	BPI002CC
		FGHIJKM	Brunei1.BPIbago
		FGHJKM	Brunei4.BPIbago
		FGJKM	Brunei5.BPIbago
7. CGR5110	16	AJL	NativePol
		AJ	Nativ10IdioKul
		AH	ChanIdioKul
		ACFGHIJKL	ArancilloB.Balindog
		ABCDL	Cobb1USM
		ABCDEFGHIJL	MonthongBPI
		ABCEFGHIL	MonthongUSM
		ABCFGHIJL	AlconFancyUSM
		ABCDFGHIJKL	AlconFancyMak
		ABCDGHIJKL	UmaliBPI
		AB	UnIDPol
		ABCDGHL	MDur88BPI
		ABCDEFGHL	Hybrid2BPI
		HKL	DuyayaUSM
		ABCDHKL	KarnYaoBPI.Bal
		HK	Duraya(But)BPIBago
		ABEFGHK	GD69USM
		AHK	MaridagaoBpiBal
8. DZC01	19	CEFH	NativePol
		BDEFH	NativBanga
		ADEFH	NativIdioFarmKul
		ABEFH	NativMasilimKul
		DE	Chanee2815BPI
		EE	ChaneePol
		CDEF	Puyat2BPI
		CDEH	AlconFancyUSM
		BCDE	DuyayaBPI
		BCDEFH	KarnYaoBPI
		AA	MadonnaUSM
		ABE	ChaneeIdioKul
		ABCDFGH	ArancilloZam
		CE	#3KSC
		BCEFH	16FS
		CEF	3KSC
		EFGH	Brunei1.BPIbago
		ACEFH	Brunei5.BPIbago
		ABDEFH	Lacson1.BPIbago
9. CGR5100	18	ABCDF	ChaneeUSM
		ABDFGKL	ChaneePol
		ADFG	Puyat2BPI
		DEGKL	D101.B.BPI
		ABDGKL	Cobb1BPI
		ACDEFGKL	Cobb1USM
		DEFGKL	ObozaBPI
		DFG	SuspMonthPol
		DGK	RedPrunTSF
		ADF	BPI006CC
		DEF	#4FS
		ABCDEFKL	14FS
		BCDFKL	#3KSC
		ABCDEFGKL	BPI027BK

Loci	No. of Unique Alleles	Genotypes	Clones
		ADFKL	#15FS
		DFGHKL	3KSC
		ADEFIJKL	Brunei2.BPIbago
		DEKL	Brunei4.BPIbago
10. DZA01	7	ABDEF	NativS.milagrosaTamp
		BCFG	D101.USM
		CC	CobbBanga
		FG	DuyayaUSM
		BB	2KSC
		BCDEF	14FS
		ABCFG	Lacson1.BPIbago
11. CGR5238	16	ADE	Native7SquibelKul
		ADEHI	ArancilloUSM
		ABCDEFGHI	Puyat1BPI
		GHI	Puyat2BPI
		AHI	Cobb1BPI
		ADEGH	MonthongUSM
		ABCDFH	MDur88USM
		GH	DuyayaBPI
		ABCDEFGH	SuspNanam4Pol
		DH	Hyb2BPIbago
		BCDH	DES218USM
		CC	RajahKunyitUSM
		BCH	ChaneeIdioKul
		ACH	ArancilloZam
		ABDEFH	Brunei1.BPIbago
		ABDEH	Brunei2.BPIbago
12. DZCAG01	9	ABCDEFGH	ArancilloUSM
		CDEFG	MonthongLacsonBPI
		ABCFG	DuriogravBPI
		CC	Mamer806.BPIbago
		ABCDEG	DES218USM
		ABCEFG	BPI16AC
		ABEFG	#9FS
		ABE	8FS
		AD	ThornlessBPIbago
13. CGR6729	22	BC	ChanIdioKul
		DD	Chanee2815BPI
		ABDEH	ChaneeBPI
		HH	MonthongUSM
		II	CobbMak
		HI	MonthongBPI
		ABCDEFG	MonthongLacsonBPI
		CEH	AlconFancyBPI
		BCEH	AlconFancyUSM
		ABCEH	AlconFancyMak
		ABDEFGHI	MDur88USM
		ABDEGHI	UmaliMonthUSM
		ACDEFGHI	DuyayaUSM
		CD	At/mamBPIBal.A
		ABDEF	SorianoBPIbago
		ACDEFGH	4FS
		ABCEGHI	#17$_2$FS
		ACEH	#15FS
		ABCDEGH	16FS
		BCDEH	Brunei1.BPIbago
		BCDE	Brunei4.BPIbago
		CDEH	Lacson1.BPIbago
14. CGR5117	23	BCDEG	NativS.milagrosaTamp
		CDEG	NativePol
		CC	Native2Kul
		BC	CobbBanga
		BCDEFGHI	MonthongUSM
		ABCEFGH	MDur88USM
		FG	UmaliMonthUSM
		CEGH	LacsonChaneeBPI
		ABCDEGH	At/mamBPIBal.A
		AH	Hyb1BPIbago
		EG	UnID.2Line1Pol
		EE	RedPrunTSF
		BCDEGHIK	TSF2
		BCGH	ThornlessZam
		CDGH	12FS
		ABCDGHL	8FS
		ABCDEGIK	BPI019BK
		ABG	BPI005CC
		BB	16FS
		BGH	BPI013AC
		ABCDEGI	Brunei4.BPIbago
		ABCDEGHI	Brunei5.BPIbago
		ABCDHI	ThornlessBPIbago
15. CGR5136	10	ABGHI	NativIdioFarmKul
		GI	ChanIdioKul
		ABDEG	ArancilloBPI
		ADGI	ArancilloBanga
		ABCDGHI	DuyayaUSM
		ABDHI	KarnYaoBPI
		EG	SuspNanam3.Pol
		AG	PuyatxMdurP2USM
		DD	MonthongZam
		ADEFGHI	Brunei1.BPIbago
16. CGR5141	20	BCE	NativePol
		BE	Native7SquibelKul
		DEF	NativBanga
		FH	ArancilloKul
		EG	ChaneeUSM
		BDF	Puyat1BPI
		CDEFGH	D101.B.BPI
		CC	MonthongTSF
		CDEFG	LacsonChaneeBPI
		ABCDEG	SuspNanam4Pol
		BCEFGH	DvoSelMasilimKul
		EE	ThornlessZam
		BCFGH	BPI022BK
		BCDF	#3BPIbago
		BCEF	UnID.BPIbago
		ABCEF	BPI16AC
		BCF	13FS
		BCDEF	#3KSC
		ACDEFGH	#15FS
		BCDG	Brunei1.BPIbago
17. CGR5030	36	ADEGHI	NativS.milagrosaTamp
		AE	NativeKul
		AD	Native2Kul
		ACEFGHI	NativeTamp
		ACDEFGHIL	Native7SquibelKul
		ADEFGHIKL	ArancilloUSM
		HIKL	ArancilloBPI
		AFGH	ChaneePol
		ABGH	ChaneeMasilimKul
		ABDEFGHIK	Puyat1BPI
		ADEFGHIK	Puyat2BPI
		AEFGHIK	PuyatUSM
		AEFH	PuyatKul
		AEFGH	PuyatTSF
		GL	MonthongKuL
		EFGHL	MonthongLacsonBPI
		ACDEFGH	UmaliBPI
		ACDEFH	UmaliMahF.Kul
		ACDEFGHIK	LacsonChaneeBPI
		ABDEFGHIKL	ObozaBPI
		HIL	ObozaUSM

Loci	No. of Unique Alleles	Genotypes	Clones
		DHI	GD69.USM
		AEFHI	SuspNanamPol
		AFI	At/mamBPIBal.A
		HIK	SuspMonthPol
		ACDEF	Pal2.BPIbago
		ADEFI	MonthBPIbago
		AEFIK	DES218USM
		ACDEHI	UnID.1Pol
		AHI	UnID.2Kul
		ADHI	UnID.3Kul
		CEF	MonthongZam
		ABCDEFGHK	#5FO
		ACEF	BPI007CC
		ACEFI	#3KSC
		ACDEH	3KSC
18. CGR5091	24	AC	NativeTamp
		ABCEHI	ChaneeUSM
		FHI	Cobb2.BPI
		EFHI	Cobb1USM
		EHI	MonthongBPI
		ABCDFHI	AlconFancyUSM
		AHI	AlconFancyMak
		CC	ChaneeYolF.Tupi
		ACDHI	KarnYaoBPI.Bal
		ACDFHI	GD69.Mak
		AA	SuspNanam3.Pol
		FF	MonthBPIbago
		ABCHI	PuyatxMdurP2USM
		BCDEHI	KradumtongUSM
		BDHI	RajahKunyitUSM
		ABCG	BPI022BK
		BCJ	BPI006CC
		ACIJ	12FS
		BC	#3BPIbago
		BIJ	8FS
		ABCFIJ	10FS
		ABCGIJ	#3KSC
		ABCHIJ	#17$_2$FS
		BCEHIJ	Brunei1.BPIbago
19. DZGCC01	1	ABCDEF	#17$_2$FS
20. DPL0725	2	BDHI	Nativ9IdioKul
		ABI	Nativ11IdioKul
21. DPL0790	19	FF	NativeTamp
		ABCDH	ChanIdioKul
		BDF	ArancilloBanga
		CI	ArancilloKul
		DGH	PuyatUSM
		ABCFHI	PuyatMak
		CDFG	Cobb1USM
		AA	CobbBanga
		CDG	MonthongUSM
		BCFGH	MDur88BPI
		ABCDEFH	Mdur88USM
		ACDFGH	KanYaoUSM
		CDH	SuspNanam1.Pol
		ACDF	MadonnaUSM
		BCDEF	RajahKunyitUSM
		ABCDF	MaridagaoBpiBal
		BCEFG	UnID.3BPIbago
		BDG	BPI16AC
		ABCD	BPI013AC
22. SHIN1621	37	ADIJK	Native2Kul
		ACIJK	Nativ4SquibelKul
		BDFGIJK	Nativ12IdioKul
		BCDFGIJK	ArancilloUSM
		CDFIJ	ChaneeKul
		CDFIJK	Puyat1BPI
		CEIJ	PuyatTSF
		BDEIJ	D101.B.BPI
		BCDEIJ	D.101.B.BPI
		EIJ	CobbMak
		ADEGHIJK	AlconFancyUSM
		DEGIJ	MDur88BPI
		ADEFGHIJK	UmaliMonthUSM
		EHIJK	LacsonChaneeBPI
		EIJK	LacsonUnoUSM
		DEFHIJK	DuyayaUSM
		ADFHIJK	KarnYaoBPI
		ADEFHIJK	GD69.USM
		AEJ	Mamer806.BPIbago
		EJ	At/mam806Bal.B
		AEFGJ	Hyb2BPIbago
		BJ	Pal1.BPIbago
		ADEFGJ	DES218USM
		AIJ	PuyatxD24P22USM
		ADEIJK	RajahKunyitUSM
		ADIJ	MaridagaoBpiBal
		KK	UnID.TSF
		ADEGIJ	ArancilloZam
		ABCDEFIJK	BPI006CC
		ABDEFGIJK	21FS
		ABCDEFGIJK	#3BPIbago
		AEFGHIJK	UnID.BPIbago
		ABCDFGHIJK	BPI16AC
		HIJK	6FS
		ACEFIJK	Brunei3.BPIbago
		AFIJK	Brunei4.BPIbago
		AEFIJK	Lacson1.BPIbago
		AEGIJK	ThornlessBPIbago
23. DPL0912	45	BIJ	NativS.milagrosaTamp
		BG	Native2Kul
		ACEI	Nativ4SquibelKul
		BDFGHIJ	Nativ10IdioKul
		BCD	Nativ12IdioKul
		BC	ArancilloUSM
		BB	ArancilloC.Balindog
		CDEGH	ArancilloKul
		BF	Chanee2815BPI
		HI	ChaneePol
		IJ	ChaneeMasilimKul
		ABCDEGHIJ	ChaneeKul
		ABCEHI	Puyat2BPI
		DHI	Puyat2Mak
		ABCEI	PuyatTSF
		BCIK	Cobb1USM
		BCDGHI	MonthongBPI
		BCGHI	AlconFancyMak
		BHI	MDur88BPI
		BCFGHI	MdurKul
		GI	UmaliMahF.Kul
		BCEG	LacsonUnoUSM
		BCDEGHIK	DuyayaUSM
		ABCDHI	SuspNanamPol
		ACDI	SuspNanam4Pol
		AB	DuriogravBPI
		ABCHI	Pal1.BPIbago
		BCGJ	DurianBatoUSM
		BCFGHJ	PuyatxMdurP2USM
		ABCDEFGH	PuyatxD24P22USM
		ABCDEFGHIJ	KradumtongUSM
		ABFGHI	SolomonPol
		ABCDE	TSF2

Loci	No. of Unique Alleles	Genotypes	Clones
		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
24. DPL0564	24	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
		ACDEFGHI	KarnYaoBPI.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.

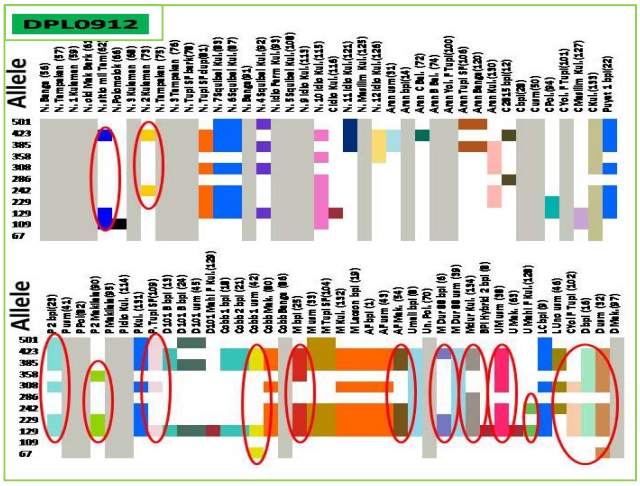

Fig. 1 Sample of DNA profile of the durian cultivars detected by SSR primer DPL0912

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.

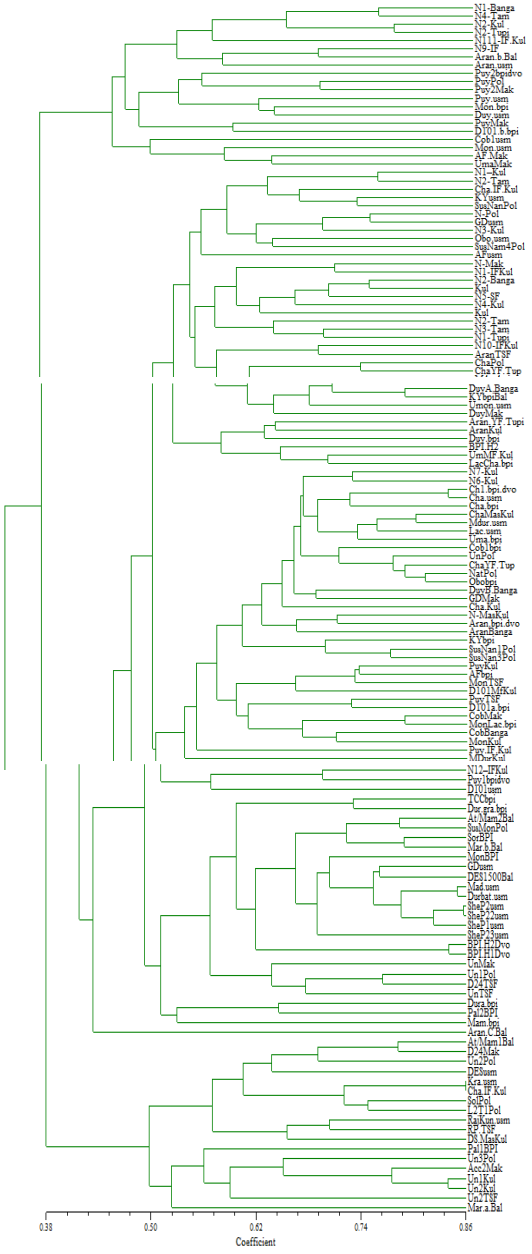

Fig. 2 Dendrogram of 187 Durian clones constructed by NTSYSpc version 2.10z and clustered by UPGMA [4] based on 21 SSR markers

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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