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 procedures 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 procedures 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 of the microsatellites development process with the optimization of the microsatellites development process.

**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...
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 from a set of SSR primers. Such unique alleles that were generated were named in reference to DNA fragments generated from a set of SSR primers. Such unique alleles that were generated were named in reference to DNA fragments generated from a set of SSR primers.

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 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
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

<table>
<thead>
<tr>
<th>Durian Cultivars /Accessions collected</th>
<th>Location</th>
</tr>
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<tbody>
<tr>
<td>1 Native Banga</td>
<td>Banga, South Cotabato</td>
</tr>
<tr>
<td>2 Native 1 Tampakan</td>
<td>Tampakan, South Cotabato</td>
</tr>
<tr>
<td>3 Native Sitio Milagrosa Tampakan</td>
<td>Sitio Milagrosa Tampakan, South Cotabato</td>
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<td>4 Native Polomolok Farm 1</td>
<td>Polomolok Farm 1, South Cotabato</td>
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<td>5 Native 2 Tampakan</td>
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</tr>
<tr>
<td>6 Native 3 Tampakan</td>
<td>Tampakan, South Cotabato</td>
</tr>
<tr>
<td>7 Native Tupi Seed Farm (Bark)</td>
<td>Tupi Seed Farm, South Cotabato</td>
</tr>
<tr>
<td>8 Native Tupi Seed Farm (Duplicate)</td>
<td>Tupi Seed Farm, South Cotabato</td>
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<tr>
<td>9 Native Banga</td>
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<tr>
<td>10 Arancillo Yolanda Farm Tupi</td>
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<td>11 Arancillo Tupi Seed Farm</td>
<td>Tupi Seed Farm, South Cotabato</td>
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<td>12 Arancillo Banga</td>
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<td>15 Puyat Polomolok Farm 1</td>
<td>Polomolok Farm 1, South Cotabato</td>
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<td>16 Puyat Tupi Seed Farm</td>
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<td>17 Cobb Banga</td>
<td>Banga, South Cotabato</td>
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<td>18 Monthong Tupi Seed Farm</td>
<td>Tupi Seed Farm, South Cotabato</td>
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<td>19 Unidentified Polomolok</td>
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<tr>
<td>20 Chance 3 Yolanda Farm 2</td>
<td>Yolanda Farm 2 Tupi, South Cotabato</td>
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<td>25 Suspected Nanam Polomolok 4</td>
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<td>26 Suspected Nanam Polomolok</td>
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</tr>
<tr>
<td>27 Suspected Nanam 3 Polomolok</td>
<td>Polomolok, South Cotabato</td>
</tr>
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<td>28 Suspected Nanam Polomolok</td>
<td>Polomolok, South Cotabato</td>
</tr>
</tbody>
</table>
Durian Cultivars/Accessions collected | Location
---|---
29 Suspected Monthong | Polomolok, South Cotabato
30 Soloman 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, 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 Old Native 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 Cob 1 USM | USM, Kabacan, North Cotabato
49 Cob 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 Ataærine/Mamer BPI Balindog | Balindog, Kidapawan City
65 Ataærine/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 Payut x Mdur P2 USM | USM, Kabacan, North Cotabato
72 Payut x Mdur P1 USM | USM, Kabacan, North Cotabato
73 Payut x D24 P22 USM | USM, Kabacan, North Cotabato
74 Payut x D24 P23 USM | USM, Kabacan, North Cotabato
75 Kruamnoong USM | USM, Kabacan, North Cotabato
76 Rajah Kuyit USM | USM, Kabacan, North Cotabato
77 Unidentified Makilala | Makilala, North Cotabato
78 Maridagao A 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 Squirrel Kulaman | Kulaman Squirrel Farm, Sultan Kudarat
89 Native 6 Squirrel Kulaman | Kulaman Squirrel Farm, Sultan Kudarat
90 Native 4 Squirrel Kulaman | Kulaman, Sultan Kudarat
91 Native Idro Farm Kulaman | Kulaman Farm, Sultan Kudarat
92 Native 5 Squibel Kulaman | Kulaman Squibel Farm, Sultan Kudarat
93 Native 9 Idro Fram Kulaman | Kulaman Idro Farm, Sultan Kudarat
94 Native 10 Idro Farm Kulaman | Kulaman Idro Farm, Sultan Kudarat
95 Native 11 Idro Farm Kulaman | Kulaman Idro Farm, Sultan Kudarat
96 Native 1 Masilim Farm Kulaman | Kulaman Masilim Farm, Sultan Kudarat
97 Native 12 Idro Farm Kulaman | Kulaman Idro Farm, Sultan Kudarat
98 Arancillo Kulaman | Kulaman, Sultan Kudarat
99 Chance Masilim Farm Kulaman | Kulaman Masilim Farm, Sultan Kudarat
100 Chanee Kulaman | Kulaman, Sultan Kudarat
101 Puyat Idro Farm Kulaman | Kulaman Idro 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 Chance Idro Farm Kulaman | Kulaman Idro Farm, Sultan Kudarat
107 Davao Selection Masilim Farm Kulaman | Kulaman Masilim Farm, Sultan Kudarat
108 Unidentified 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 Bagu Oshiro, Davao City
114 Chanee 2815 BPI | BPI Bagu Oshiro, Davao City
115 Chanee BPI | BPI Bagu Oshiro, Davao City
116 D 101 A BPI | BPI Bagu Oshiro, Davao City
117 D 101 B BPI | BPI Bagu Oshiro, Davao City
118 Cob 1 BPI | BPI Bagu Oshiro, Davao City
119 Cob 2 BPI | BPI Bagu Oshiro, Davao City
120 Monthong BPI | BPI Bagu Oshiro, Davao City
121 Monthong Lacson BPI | BPI Bagu Oshiro, Davao City
122 Alcon Fancy BPI | BPI Bagu Oshiro, Davao City
123 Umali BPI | BPI Bagu Oshiro, Davao City
124 Mdur 88 BPI | BPI Bagu Oshiro, Davao City
125 BPI Hybrid 2 | BPI Bagu Oshiro, Davao City
126 Lacsac Chance BPI | BPI Bagu Oshiro, Davao City
127 Duyaya BPI | BPI Bagu Oshiro, Davao City
128 Karn Yao BPI | BPI Bagu Oshiro, Davao City
129 Oboza BPI | BPI Bagu Oshiro, Davao City
130 Tang Chai Churot BPI | BPI Bagu Oshiro, Davao City
131 Durio Graveolens BPI | BPI Bagu Oshiro, Davao City
132 Duraya (Butuan) BPI | BPI Bagu Oshiro, Davao City
133 Mamer 806 BPI | BPI Bagu Oshiro, Davao City
134 BPI Hybrid 2 BPI | BPI Bagu Oshiro, Davao City
135 BPI Hybrid 1 BPI | BPI Bagu Oshiro, Davao City
136 Palawan 1 BPI | BPI Bagu Oshiro, Davao City
137 Palawan 2 BPI | BPI Bagu Oshiro, Davao City
138 Monthong BPI | BPI Bagu Oshiro, Davao City
139 D-24 | BPI Bagu Oshiro, Davao City
140 Duraya | BPI Bagu Oshiro, Davao City
141 Sorrano BPI | BPI Bagu Oshiro, Davao City
142 BPI 004 CC | BPI Bagu Oshiro, Davao City
143 BPI 006 CC | BPI Bagu Oshiro, 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
<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequence</th>
<th>Allele size range</th>
<th>No. of alleles</th>
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<tr>
<td>1. CRG5334</td>
<td>F-5'-AGC ATT GGC CCT TGT TT-3' R-3'-ACT TGC CAC GTT CAT CAC AA-5'</td>
<td>1125-50</td>
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<tr>
<td>2. CRG6723</td>
<td>F-5'-CTA GGT CGA TGC TCT GC-3' R-3'-CGC ATC TAT CCG AGA AGC TG-5'</td>
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<tr>
<td>3. CRG6729</td>
<td>F-5'-AGT GCC AGA GAT ACG GGA AA-3' R-3'-TGC AGG AGA ATG GAA GCT CT-5'</td>
<td>501-168</td>
<td>109</td>
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<tr>
<td>4. CRG6987</td>
<td>F-5'-CTG GTC TGC TCC TCC TCA C-3' R-3'-CTG TGC TGA CCA AGA AGA GC-5'</td>
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<td>5. DPL0056</td>
<td>F-5'-GTT AAT GCT CTC CCT CTC CCT C-5' R-3'-TAA GCC TAA GAG GGC TGC AAC AA-5'</td>
<td>490-124</td>
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<tr>
<td>6. DPL0072</td>
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<td>490-130</td>
<td>146</td>
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<tr>
<td>7. DPL0079</td>
<td>F-5'-ACA ATG GGC GAT TGG ATT C-3' R-3'-TTC CAA GTG TCA CCC TCT CAC-5'</td>
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<tr>
<td>8. DPL0911</td>
<td>F-5'-ACA GGT GAC GAT TCG TGG AG-3'</td>
<td>480-85</td>
<td>123</td>
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**Durian Cultivars /Accessions**

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<tr>
<th>No.</th>
<th>Primers</th>
<th>Location</th>
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</thead>
<tbody>
<tr>
<td>152</td>
<td>8 FS</td>
<td>Francisco Sasing, Calinan, Davao City</td>
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<td>154</td>
<td>11 FS</td>
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<td>155</td>
<td>10 FS</td>
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<tr>
<td>156</td>
<td>2 KSC</td>
<td>Kapitana, Sirib, Calinan, Davao City</td>
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<td>14 FS</td>
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<td>158</td>
<td>BPI 003 CC</td>
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<td>BPI 007 CC</td>
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<td># 17, FS</td>
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<td>170</td>
<td>Brunei 1 BPI</td>
<td>BPI Bago Oshiro, Davao City</td>
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<td>Brunei 3 BPI</td>
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**TABLE II**

List of Informativ SSR Primers Screened for Their Utility in Identifying Durian Collections

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<tr>
<th>Primers</th>
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<th>Allele size range</th>
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<td>9. DPL0912</td>
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<td>10. SHIN-1574</td>
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<td>12. CRG5238</td>
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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.

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**TABLE III**

Characteristics of 29 Microsatellites Which Amplified Substantial PCR Product with 187 Durian Accessions

**TABLE IV**

Unique Alleles/Genotypes Generated by 29 SSR Markers from Each of the 187 Durian Clones Collected
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BJ | 8FS | |
ABCFIJ | 10FS | |
ABCGJ | #3KSC | |
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BCEHIJ | Brune1.BPIbago | |

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21. DPL0790 19
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DGH | PuyatUSM | ABCFHI | PuyatMak | CDFG | Cobb1USM | AA | CobbBanga | CDG | MonthongUSM | BCFGH | MDur88BPI | |
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22. SHIN1621 37
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21. DPL0790 19
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ABCDH | ChanildioKul | BDF | ArancilloBanga | CI | ArancilloKul | |
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22. SHIN1621 37
ADJJK | Native2Kul | ACJK | Native4SquibelKul | BDFGHJK | Native2Kul | BDG | BPI16AC | ABCD | BPI013AC | |
## Loci No. of Unique Alleles Genotypes Clones

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### 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 DPL 0912 detected 37 clones with unique genotypes; primer CGR 5117 detected 22 clones with unique genotypes; primer CGR 5141 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.

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.

ACKNOWLEDGEMENT

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