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SSR Markers for Fingerprinting, Hybridity Testing and Diversity Analysis of Important Varieties and Promising Lines of Papaya in the Philippines

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Simple sequence repeat (SSR) or microsatellite markers were identified for Philippine varieties of papaya for varietal identification, diversity analysis, and hybridity assessment. Fourteen SSR markers were screened using PCR for amplification and polymorphism. Six primer combinations namely, CPMET, BGAL, ACC, CPY, SSR 12 and SSR 35, were effective in fingerprinting and diversity analysis and for hybridity testing, except for CPMET. The genetic relationship of the selected papaya varieties was established based on UPGMA clustering. At 0.76 coefficient of similarity, three groups were observed, namely: Group I (Davao Solo, Maradol and Red Lady), Group II (Sinta and Cavite Special) and Group III (Morado). The heterozygosity of each locus varied from 0.38 to 0.57 with an average of 0.51. ACC, BGAL and SSR 35 markers were predominated by major alleles with ≥ 75 percent. Distinct DNA fingerprints were obtained for the six papaya cultivars, the transgenic papaya lines, backcross lines, recurring parent and hybrids. For hybridity testing, the SSR markers CPY, BGAL, SSR 12 and SSR 35 were able to discriminate the F_1 hybrid and its parents. This study shows that the identified SSR markers are effective in analyzing diversity, providing definitive fingerprints and testing hybridity.

Key words: SSR, microsatellite markers, molecular markers, papaya, DNA fingerprints, genetic diversity analysis, hybridity testing

Abbreviations: PCR – polymerase chain reaction, PIC – polymorphism information content, SSRs – simple sequence repeats

INTRODUCTION

Papaya is the fourth most important fruit commodity in the Philippines with a production of 164,913 metric tons (MT) in 2012, 166,336 MT in 2013, 172,628 MT in 2014, and 162,481 in 2016 (PSA 2017). Export increased to 5,295 MT in 2014, but decreased to 1,834 MT in 2015 and 1,588 MT in 2016 (PSA 2017). The Philippines ranked number eight in world production of papaya in 2014, representing 1.4% of the world's share (FAOSTAT 2017). The failure to increase papaya production has been attributed primarily to the ringspot virus infestation of papayas in various parts of the country (Espino and Espino 2013). In addition to its utilization as fresh and dried fruits and as an ingredient of tropical fruit cocktail and native dishes, papaya is a source of papain, an enzyme used in meat tenderizing and clearing of fruit and alcoholic beverages, and also in the production of pharmaceuticals, cosmetics, and beauty products.

In the Philippines several cultivars of papayas are commercially produced: 'Solo', 'Cavite Special', 'Morado', 'Red Lady' and 'Sinta'. Both 'Red Lady' and 'Sinta' are F_1 hybrids. 'Sinta' was developed at the Institute of Plant Breeding, College of Agriculture and Food Science, University of the Philippines Los Baños and released in 1995; it is now widely grown in the country and its seeds are exported to various Asian countries as well. The 'Solo' papaya is the primary export variety while 'Sinta', 'Cavite Special', 'Morado' and 'Red Lady' are locally consumed and used for processing.

The increasing local production and commercialization of papaya underscores the importance

of correct identification of varieties and assurance of genetic purity. The commercialization of hybrid technology depends to a large extent on the quality of the hybrid seed supplied (Tamilkumar et al. 2009), thus, parental identification and hybridity testing are of utmost importance. These serve to ensure genetic purity and protect plant breeders' rights. Traditionally, genetic purity assessment is done based on grow-out test in the field which is time-consuming and expensive. The procedure requires large land areas and the hiring of skilled personnel who often make subjective decisions (Cooke 1995). Furthermore, the morphological characteristics of hybrids could be similar to one of the parents which make visual assessment unreliable (Yashitola et al. 2002; Dongre and Parkhi 2005) and prone to errors. The use of isozyme analysis offers a solution to purity testing but its use is sometimes limited due to possible effects of environmental conditions and tissue type and requires selection of suitable isozymes for variety identification and purity testing (Liu et al. 2008). These problems in purity testing can be managed more effectively by using DNA or molecular markers.

Among DNA markers, the polymerase chain reaction (PCR)-based markers such as microsatellites, offer a more reliable purity test tool because of their abundance and even distribution throughout the genome (Akagi et al. 1996; McCouch et al. 1997; Wu and Tanksley 1993). Microsatellite DNA markers based on simple sequence repeats (SSRs) exhibit high rates of polymorphisms and co-dominant inheritance and are assayed efficiently by PCR. This makes SSRs a valuable marker for testing distinctiveness of varieties and for proprietary protection (Law et al. 1998). SSRs have been used successfully as tools for varietal identification and hybrid purity assessment in rice (Yang et al. 1994; Rongwen et al. 1995; Tamilkumar et al. 2009), tomato (Liu et al. 2007), olives (Taamalli et al. 2008), and melon (Fen et al. 2008).

Using SSR primers, Matos et al. (2013) analyzed 96 papaya accessions from seven countries and showed relationships among the papaya accessions regardless of their classification types. Asudi et al. (2013) reported that the genetic diversity of 42 papaya accessions in Kenya had great genetic similarity (0.802) using SSR primers. Ocampo Perez et al. (2007) found that a number of SSR markers in *C. papaya* showed polymorphism in *Vasconcellea* species. Recently, Oliveira et al. (2015) compared the use of minisatellite and microsatellite markers in papaya and found lower informativeness of minisatellites over microsatellites. However, this can be overcome by the less error in the genotyping and use of a larger number of loci for minisatellites. In molecular-assisted selection, the use of SSRs was shown to be effective in developing papaya lines since genotyping and selection can be done in the early generations on homozygous individuals which have the desired traits (Oliveira et al. 2010). An SSR atlas comprising 160,318 SSR markers located in genic regions has also been published to serve as an important resource by Vidal et al. (2014).

This study identified and characterized SSR markers which were used in the fingerprinting or varietal identification, hybridity testing, and genetic diversity analysis of selected papaya cultivars, accessions and promising lines from the Philippines. The identification of discriminating microsatellite markers that can assess purity and distinctiveness of the parents of Philippine papaya cultivars and lines will be of great importance for government regulators, international trade organizations, and industries for product commercialization, utilization, and for plant variety protection.

MATERIALS AND METHODS

Plant Materials

The samples of *C. papaya* used in this study include papaya cultivars, namely, 'Davao Solo', 'Morado', 'Maradol', 'Red Lady', 'Sinta' and 'Cavite Special', parental and promising lines consisting of transgenic lines of papaya with long shelf life, papaya ringspot virus (PRSV)-resistant backcross 3 and 4, F_1 hybrids of transgenic lines by BC3/BC4, inbred lines 5648 and 4172, and several others (Supplement Table 1).

Leaf samples from transgenic papaya lines of four events, namely, 137, 139, 213 and 218, and the F_1 hybrids were obtained from the BL2 screen house at the Institute of Plant Breeding (IPB), College of Agriculture and Food Science, University of the Philippines Los Baños, Laguna courtesy of the project led by Dr. Evelyn Mae Tecson-Mendoza. The leaf samples from PRSV-resistant backcrosses were obtained from the experimental area located at Barangay Paciano Rizal, Bay, Laguna, courtesy of the late Dr. Simeona V. Siar of the Fruit and Ornamental Crops Divison, IPB. Meanwhile, leaf tissue samples of Davao Solo, Morado, Maradol, Red Lady, and Sinta were obtained from the collection of Dr. Pablito M. Magdalita of the Fruit and Ornamental Crops Division, IPB. Plant tissues from the youngest true leaves were collected for genomic DNA isolation.

Genomic DNA Isolation

The protocol used for extracting papaya DNA according to Cheung et al. (1993) and Dellaporta et al. (1983), as

Supplement Table 1. Summary of papaya accessions including the commonly cultivated papaya varieties used in the study.

No.	Papaya Accessions	Description
	A. Fingerprinting Analysis	
1	Davao Solo (DS)	High quality selection with reddish-orange flesh; pear-shaped.
2	Maradol (Ma)	The fruit of Maradol variety has a red-orange skin and salmon red pulp. Originally from Cuba, this variety was quickly introduced in other countries and has become a commercially prominent cultivar (Basulto 2009).
3	Morado (Mo)	Morado is named according to its color. Because of the natural out-crossing, the plants do not produce true-to-type fruits.
4	Red Lady (RL)	A semi-dwarf type that blooms 6 mo after planting. Fruit is large and oblong. It has a star-shaped cavity and the flesh is yellowish orange.
5	Sinta (Sn)	First Philippine hybrid papaya, moderately tolerant to PRSV. It is semi-dwarf and easy to harvest.
6	Cavite Special (CS	Tolerant to PRSV; fruits are short and oblong in female plants and long shaped in bisexual plants.
	B. Hybridity Testing	
7	BC3 73-3	Female parent
8	BC3 34-14	Female parent
9	BC3 73-9	Female parent
10	BC3 73-21	Female parent
11	BC3 73-23	Female parent
12	BC3 73-25	Female parent
13	BC3 73-27	Female parent
14	BC3 73-29	Female parent
15	BC3 86-6	Female parent
16	BC4 73-14	Female parent
17	BC4 86-2	Female parent
18	T0 218.7-4	Male parent
19	T0 218.7-9	Male parent
20	T0 218.7-11	Male parent
20	T2 218.7-4-16	Male parent
22	T3 137 04 a-4-48-5	Male parent
23	T3 139 5-3-16-4	Male parent
24	T3 139 5-3-16-5	Male parent
25	T4 213 01-6-48-3	Male parent
26	T4 213 01-6-48-6	Male parent
27	T4 213 01-6-48-12	Male parent
28	T4 213 01-6-48-13	Male parent
29	T4 213 01-6-48-8	Male parent
30	T4 213 01-6-48-10	Male parent
	Hybrids	
33	BC3 86-6 x T4 01-6-48-3-12	Hermaphrodite
34	BC3 86-6 x T4 01-6-48-3-15	Hermaphrodite
35	BC3 34-14 x T3 04a-4-48-17	Hermaphrodite
36	BC3 86-6 x T4 01-6-48-3-26	Hermaphrodite
37	BC3 86-6 x T4 01-6-48-3-28	Hermaphrodite
38	BC3 86-6 x T4 01-6-48-3-30	Hermaphrodite
39	BC3 86-6 x T4 01-6-48-3-32	Hermaphrodite
40	BC3 86-6 x T4 01-6-48-3-48	Hermaphrodite
41	BC3 86-6 x T4 01-6-48-3-72	Hermaphrodite
42	BC3 86-6 x T4 01-6-48-3-79	Hermaphrodite
43	BC3 86-6 x T4 01-6-48-3-95	Hermaphrodite
44	BC3 86-6 x T4 01-6-48-3-101	Hermaphrodite
45	BC3 86-6 x T4 01-6-48-3-103	Hermaphrodite
46	BC3 86-6 x T4 01-6-48-3-104	Hermaphrodite
47	BC3 86-6 x T4 01-6-48-3-105	Hermaphrodite
48	BC3 79-29 x T2 218.7-4-16-7	Pistillate
49	BC3 73-29 x T2 218.7-4-16-18	Pistillate
50	BC3 73-29 x T2 208-7-4-16-20	Pistillate
51	BC3 73-29 x T2 208-7-4-16-23	Pistillate
52	BC3 86-6 x T4 01-6-48-3-23	Pistillate
53	BC3 86-6 x T4 01-6-48-3-30	Pistillate
54	BC3 86-6 x T4 01-6-48-3-52	Pistillate
55	BC3 86-6 x T4 01-6-48-3-68	Pistillate
56	BC3 73-29 x T2 208-7-4-16-78	Pistillate
57	BC3 86-6 x T4 01-6-48-3-81	Pistillate
58	BC3 73-29 x T2 208-7-4-16-90	Pistillate
59	BC3 86-6 x T4 01-6-48-3-92	Pistillate
60	BC3 86-6 x T4 01-6-48-3-97	Pistillate
61	BC3 86-6 x T4 01-6-48-3-98	Pistillate
62	BC3 86-6 x T4 01-6-48-3-106	Pistillate
63	BC3 86-6 x T4 01-6-48-3-120	Pistillate
64	BC3 86-6 x T4 01-6-48-3-122	Hermaphrodite/ Pistillate
65	BC3 86-6 x T4 01-6-48-3-127	Hermaphrodite/ Pistillate
	Other samples	
66	Sinta	Hybrid
68	5648 (Cariflora)	Female parent
69	4172 (Cavite Special)	Male parent
70	BC_5	Staminate
71	5648	Staminate
72	Davao Solo	Staminate
73	F_1 hybrids	female and hermaphrodite source

modified by Angeles et al. (2005), involved cell tissue disruption, membrane lysis, and dissociation of proteins from DNA, removal of proteins and other DNA contaminants, and DNA precipitation.

DNA from individual samples was dissolved in sterile nanopure water, run on one percent agarose gel for quality check, and quantified using spectrophotometer. High amounts of DNA obtained ranged from 14 to 25 μg/mL. Aliquot samples from the stock were diluted with sterile nanopure water to have a uniform concentration of 50 ng/μL and 100 ng/μL for easier handling and efficient PCR amplification.

PCR Screening

A set of four microsatellite markers for *Carica papaya* L. were identified by data mining using sequence information from Genbank (http://www.ncbi.nlm.nih.gov) and additional SSR markers were obtained from the papaya draft genome (Ming et al. 2008). DNA sequences were searched using the EMBL (http://www.ebi.ac.uk/embl) and Genbank databases. Primer sequences were generated following gene sequences search, alignment, and tandem repeat analysis. Tandem Repeat Finder program (https://tandem.bu.edu/trf/trf.html) was used for SSR mining in the given sequences (Benson 1999). Flanking regions of hypervariable SSRs found in papaya sequences were the basis for primer design. These primers were synthesized by Invitrogen (Singapore). Each primer pair was subjected to Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) to determine the primer pair specificity and amplification targets. Amplification targets were checked across plants (taxid: 3193). Table 1 shows the summary of the microsatellite markers for *Carica papaya* used in this study.

Fourteen SSR primers were tested for amplification and polymorphism in all papaya samples. PCR was carried out in a Bio-Rad Thermal iCycler using the optimized conditions for amplification described below. The reaction mixtures contained DNA (50–100 ng/μL), 10x PCR buffer, 10 mM dNTPs, 50 mM MgCl2, 10 μM each of forward and reverse primers, and 1.0 U Taq polymerase (Vivantis Technologies, CA, USA). PCR cycling for all primers consisted of 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing temperature of 50 °C for ACC, 53°C for CPY, 54°C for BGAL, and CPMET, and 55 °C for SSR 12 and SSR 35, respectively, for 1 min, and again for all primers, extension for 72°C for 2 min, and a final extension at 72°C for 5 min. The PCR products were electrophoresed on 2% (w/v) agarose gels at 100 V for 20–30 min. The gels were stained with 0.005% ethidium bromide (w/v) for 1–2 min, de-stained for 15–20 min, and observed under a UV transilluminator (Gel doc 1000 single wavelength mini-transilluminator, Segrate (Milan, Italy). The 100-bp ladder (Vivantis Technologies, CA, USA) was used as a molecular weight standard to estimate the size of the microsatellite alleles.

Data Collection and Analyses

PCR amplification data were gathered and analyzed using the Quantity One analysis software (Biorad Universal Hood). Discrete bands for each SSR were determined based on their migration relative to a molecular weight size marker (100 bp DNA ladder). A genotype was assigned a null allele for a SSR locus whenever amplification product(s) was not detected in a particular genotype and marker combination. Multiple alleles were assigned to an individual genotype which produced more than two bands for a given marker. Experiments detecting null and multiple alleles were repeated from DNA isolation to PCR screening for confirmation.

PowerMarker, version 3.25 (Liu and Muse 2005) was used to calculate various parameters of genetic variability such as number of alleles per locus, major allele frequency, observed heterozygosity (H_o), expected heterozygosity (H_e), and polymorphism information content (PIC). H_o was calculated as the number of heterozygous individuals at a given locus divided by the total number of genotypes present at the locus. Gene diversity or H_e is the probability that two randomly chosen alleles from the population are different. PIC, a measure of the allelic diversity at a locus, was estimated for each of the SSR loci assayed. Major allele frequency is the frequency of the most common allele for a given locus. The equations for H_o, H_e, and PIC are given in the PowerMarker manual (http://www.powermarker.net).

The UPGMA (unweighted pair-group method using arithmetic average) was used to construct a dendrogram using distance data (Rohlf 1998).

RESULTS AND DISCUSSION

Characterization of SSR Markers and Analysis of Banding Patterns

Fourteen SSR primers were screened for patterns of amplification in six papaya cultivars and several breeding lines based on strength and clarity of banding patterns, successfully amplified PCR product of high quality, and polymorphic amplification product in the expected size. Six primer pairs amplified polymorphic and easily scorable PCR products (Table 2). ACC had the largest

Table 1. Characteristics of the 14 microsatellites markers used in the study.

Marker	Locus	Repeat Motif	GenBank Accession	Primer Sequence (5' - 3')	TA (ºC)
ACC	CPA277160	$(TA)_{9}^{imp}$ $(TA)_{6}^{imp}$	AJ277161	CCGCGGCAAGACTATCATGG TTGACTCCCGTTCTCCATCTC	51 49
BGAL	AF064786	$(TA)_{4}$ $(CA)_{10}^{imp}$	AF064786	CCGCGGGTGCAGAGACAAGCTA CCGCGGCTTAGATTGGAACCC	55 51
CPMET	CPMETTHIO	$(GT)_{5}^{imp}$	Y08322	CCGCGGGTGTCTGAGTGTG CCGCGGATATTACATGACTCTG	52 50
CPY	CPAPAP	$(TA)_{10}^{p}$	M15203	CCGCGGGGAGTATGTGGA CCGCGGGATCAGTGTAGAAGT	50 51
SSR 4	P3K6734YC2	$(TA)_{19}^{imp}$	AC239160.1	TTCCAGTCCGCATGCATATC TTTCCAAGCCACAGGAAAATG	52 51
SSR 6	P3K124YC2	$(GA)_{8}^{imp}$	AC238621.1	CATTTCGAAACTGCATCCCG TCCACGACTCCTGACGATGA	52 54
SSR 7	P3K4642YC2	$(AT)_{16}^{p}$ $(GA)_{14}^{p}$	EF661026.1	CCCATTGCTCGTCTTTCATCA TGTGGAGGTTGCAAACTGAAGAG	53 56
SSR 8	P3K8303YC2	$(TG)_{8}^{p}$	AC239153.1	CGGGGATAAAACACCTACATCTT AGCTACGTGTCATGATTGTCCA	52 54
SSR 12	P6K72CC	$(GA)_{14}^{imp}$	AC238636.1 AC238628.1	AGAGCAAATCGTCGAGCCAC CCTTCGTTTATATGCCTGCTGC	55 54
SSR 31	P6K108CC	$(ATTTT)_{5}^{p}$	AC238768.1 AC238636.1	GGGGAAAACGGTGAAAACCT TCTTCTCGGAAGAGCCACAAA	53 53
SSR 34	P3K896CC	$(GA)_{13}^{imp}$	AC239153.1 AC239204.1	CCCATCTCTTCCATCCTCAAGA GGGATGGGGAATTAAAGGGC	53 53
SSR 35	P3K168CC	$(AAT)_{24}^{imp}$ $(AAATA)_{17}^{imp}$	AC238761.2 AC238629.1	GCACAAGCGCTCTCCTTTCT TTCCCCCTCTGATCTGGTCTC	55 54
SSR 38	P6K975CC	$(AAATTTGT$ $ACTTT)_{4}^{cmp}$	EF661024.1 GQ478573.2	TGTCTGAATCTGCAGCCCAA GAAGGGGAAATTAAGCATGGGA	54 52
SSR 41	P6K595CC	$(TATTT)_{20}^{imp}$ $(TTTAT)_{17}^{imp}$	AC238599.1 AC239167.1	GCGGGGTGATTGTGAAGAAA CGTGCAATTTTCAGTCGCAG	53 53

^{imp} imperfect repeats
^{p} perfect repeats
^{cmp} compound repeats

number of alleles (6) and CPMET had the least (2). The total number of alleles was 22 and the number of banding patterns was 20. Only DNA amplification products between 50 and 500 bp were considered in the analysis. Figure 1 shows the banding patterns obtained with the six primers. The number of alleles per marker was found to be 3.7, which is better than the 3.18 obtained by Oliveira et al. (2010) but lower than the 6.6 obtained by Ocampo Perez et al. (2007) who analyzed 72 papaya accessions from 13 different geographic locations.

Gene diversity, defined as the probability that two randomly chosen alleles from the population are different from each other, varied from 0.38 (CPMET) to 0.57 (ACC, CPY and SSR 12), with an average of 0.51. The heterozygosity data showed that major alleles were prevalent in BGAL, SSR 35 and ACC markers with ≥ 75 percent. The PIC values for the six DNA markers ranged from 0.22 to 0.72 and averaged 0.56 (Table 2) comparable with the 0.19 to 0.69 values obtained by Matos et al. (2013) also for SSR markers in *C. papaya*. PIC values of >0.7 are considered highly informative while a value of 0.44 is considered moderately informative (Anderson et al. 1993). The relatively high level of informativeness of the SSR markers indicates their capability to quantify genetic diversity and identify different genotypes of papaya. Gupta et al. (1996) showed that SSR loci for a number of core repeat units are highly polymorphic between species and more importantly, between individuals within species and populations. There is a good linear relationship between the number of alleles detected at a locus and the length of the microsatellite array. Thus, the larger the repeat number in a microsatellite, the larger is the number of alleles detected.

The H_e values of the six SSR markers ranged from 0.14 to 0.30, with an average of 0.25 (Table 2), which is quite low compared with those observed by other authors which ranged from 0.09 to 0.76, with an average of 0.42 (Oliveira et al. 2015) in the genetic analysis of papaya with microsatellite markers. The results of genetic diversity in the present study can be accounted

Table 2. DNA amplification products, allele number, number of banding patterns, gene diversity, heterozygosity, and informativeness (PIC) values of the six SSR markers.

SSR Marker	DNA Amplification Products (bp)	No. of Alleles	No. of Banding Patterns	Gene Diversity	Major Allele Frequency	Heterozygosity	PIC
ACC	150-500	6	4	0.57	0.86	0.30	0.22
BGAL	150-500	3	3	0.50	0.75	0.14	0.70
CPMET	100-200	2	2	0.38	0.21	0.28	0.44
CPY	100-500	3	4	0.57	0.25	0.24	0.60
SSR 12	50-500	4	4	0.57	0.14	0.28	0.72
SSR 35	100-500	4	3	0.44	0.79	0.28	0.69
Total		22	20				
Mean		3.67	3.33	0.51		0.25	0.56

for by the smaller number of papaya accessions used as well as the fact that most of the accessions are gynodioecious, resulting in high rate of self-fertilization and a consequent reduction in heterozygosity.

DNA Fingerprinting and Diversity Analysis

DNA fingerprints of the different cultivars, parental and experimental lines were obtained. The DNA fingerprints of Davao Solo, Maradol, Morado and line 5648 are 123213, 122233, 123141, 121211, respectively (Table 3). The number for each fingerprint refers to the banding patterns being represented for each primers, CPMET, BGAL, ACC, CPY, SSR 12 and SSR 35 analyzed from 2 to 4 number of papaya samples used in the study (Fig. 1). Fingerprint results were more distinct and informative using six SSR primer pairs.

Furthermore, the DNA markers were able to distinguish the hybrid papayas resulting from crossing backcross 3 (BC3) and transgenic papaya (T4) together with other progenitors (Table 3), making them potential markers for hybridity testing. BC3 x T4 has 221312 as DNA fingerprint while BC3 and T4 have the DNA fingerprints of 221221 and 221131, respectively. PCR amplification results consistently showed discrete band products.

The genetic diversity analysis of the six papaya cultivars, Davao Solo (DS), Maradol (Ma), Red Lady (RL), Sinta (Sn), Cavite Special (CS) and Morado (Mo) was determined. Based on sequential agglomerative hierarchical nested cluster analysis using unweighted pair group method arithmetic, three groups were observed (Fig. 2) at 0.76 coefficient of similarity, namely: Group I (Davao Solo, Maradol and Red Lady), Group II (Sinta and Cavite Special) and Group III (Morado). However, all six accessions ordinated into different clusters at 0.86 similarity coefficient, indicating a high degree of genetic diversity. Results showed that Morado was the most diverse group from the rest of the commercial papaya varieties analyzed using the five SSR markers. These markers were also able to cluster together Sinta and Cavite Special. This result establishes the validity of its relatedness since Sinta is a hybrid between 5648 (Cariflora) and 4172 (Cavite Special). Davao Solo, Maradol and Red Lady, on the other hand, were clustered together at 0.80 coefficient of similarity, which may be associated with its fruit characteristics. Davao Solo has reddish-orange flesh while Maradol variety has a red-orange skin and salmon red pulp and Red Lady has yellowish orange flesh. The above results serve as a

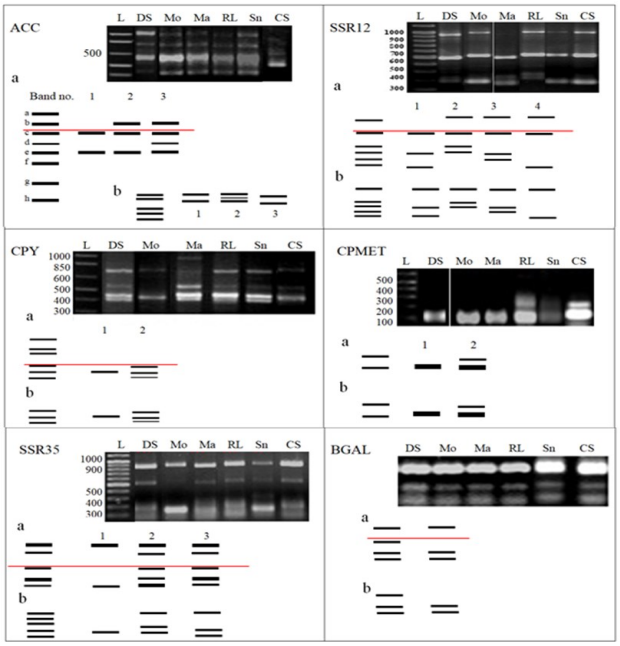

Fig. 1. SSR amplification using ACC, CPMET, BGAL, CPY, SSR 12 and SSR 35 used in *C. papaya* varieties Davao Solo (DS), Maradol (Ma), Morado (Mo), Red Lady (RL), Sinta (Sn), and Cavite Special (CS): (a) Actual banding patterns obtained; (b) Diagrammatic representation of the banding patterns of amplification products between 50 to 500 bp. Red line shows cut off of bands at 500 bp.

Table 3. Summary of DNA fingerprints generated from the different papaya genotypes using 6 SSR primers.

Reference No.	Entry (No. of Samples)	Banding Pattern					
		CPMET	BGAL	ACC	CPY	CpCdm SSR 12	CpCdm SSR 35
1	Davao Solo (2-3)	1	2	3	2	1	3
4	Maradol (2-3)	1	2	2	2	3	3
7	Morado (2-4)	1	2	3	1	4	1
10	Red Lady (2-3)	2	2	2	2	2	3
13	Sinta (2-3)	2	2	3	2	4	2
16	Cavite Special (2-3)	2	2	1	2	4	3
19	T_3 04a-4-48	2	2	1	1	2	1
20	T_4 01-6-48	2	2	1	1	3	1
21	BC_3	2	2	1	2	2	1
22	BC_4	2	2	1	2	3	1
23	$BC_3 \times T_4$	2	2	1	3	1	2
24	4172	2	2	1	2	1	1
25	5648	1	2	1	2	1	1

useful guide to breeders and genebank managers when designing crosses and enhancing access on these plant genetic resources.

Hybridity Testing

Parental identification and hybridity testing assume utmost importance in protecting plant breeders' rights and ensuring genetic purity (Tamilkumar et al. 2009). Morphological characteristics of hybrids are normally similar to one of the parents which make visual assessment more difficult (Yashitola et al. 2002; Dongre and Parkhi 2005). These limitations can be managed effectively by using molecular markers.

Among the SSR markers tested, CPY (papain) was found to discriminate between hybrids and progenitors including the control, hybrid Sinta (Fig. 3). The transgenic lines (Davao Solo) exhibited the 550 bp and 600 bp bands while the backcrosses exhibited five bands at 550 bp, 600 bp, 700 bp, 850 bp and 950 bp (Fig. 3a). Figure 3b shows 31 lines exhibiting the hybridity bands. The resulting F_1 hybrids had 550 bp, 600 bp, 700 bp and 950 bp prominent bands with an 850 bp faint band product. The intensity of the 550 bp and 600 bp bands in the F_1 hybrids was also greater than the intensity in the two parents. The inbred line 5648, which is the recurrent parent of the backcrosses, had band products with molecular weights of 550 bp, 600 bp, 700 bp, 850 bp and 950 bp.

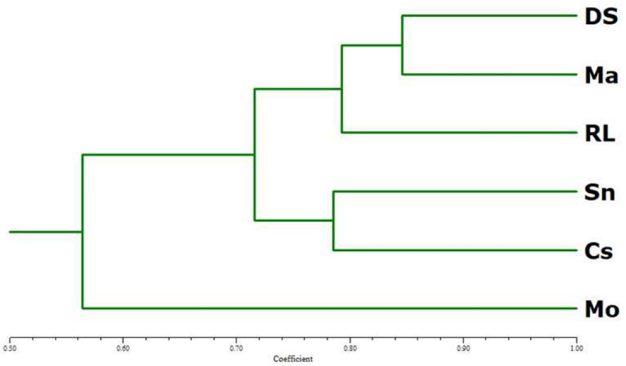

Fig. 2. Dendrogram produced by sequential agglomerative hierarchical nested (SAHN) cluster analysis using coefficient of similarity based on electrophoretic separation of DNA fragments: Davao Solo (DS), Maradol (Ma), Red Lady (RL), Sinta (Sn), Cavite Special (CS), and Morado (Mo).

Noticeably, the transgenic papayas had more intense bands at 550 bp and 600 bp compared with the backcross progenies. The intensity of the bands that were amplified in transgenic hybrids with delayed ripening trait can be used as an indication of successful introgression.

For Sinta papaya, both parents 4172 and 5648 had intense bands at 650 bp; 4172 had greater band intensity at 150 while 5648 had greater band intensity at 900 to 1000 bp (Fig. 3c). The hybrid Sinta showed pronounced bands at 900–1000 bp, 650 bp and 150 bp, indicating contributions from each parent.

Figure 4 shows the hybridity bands formed using four other primers (BGAL, CPMET, SSR12 and SSR35). BGAL primers generated two band products with molecular weights 300 bp and 100 bp. Noticeably, the hybrid obtained the 100 bp amplicon from both parents while the other band (300 bp) came from T4 only. Using the SSR 35 primers, the hybrid exhibited the 200 bp and 850 bp bands, the latter band coming from T4 while both parents had the 200 bp band. Using the SSR 12 primers,

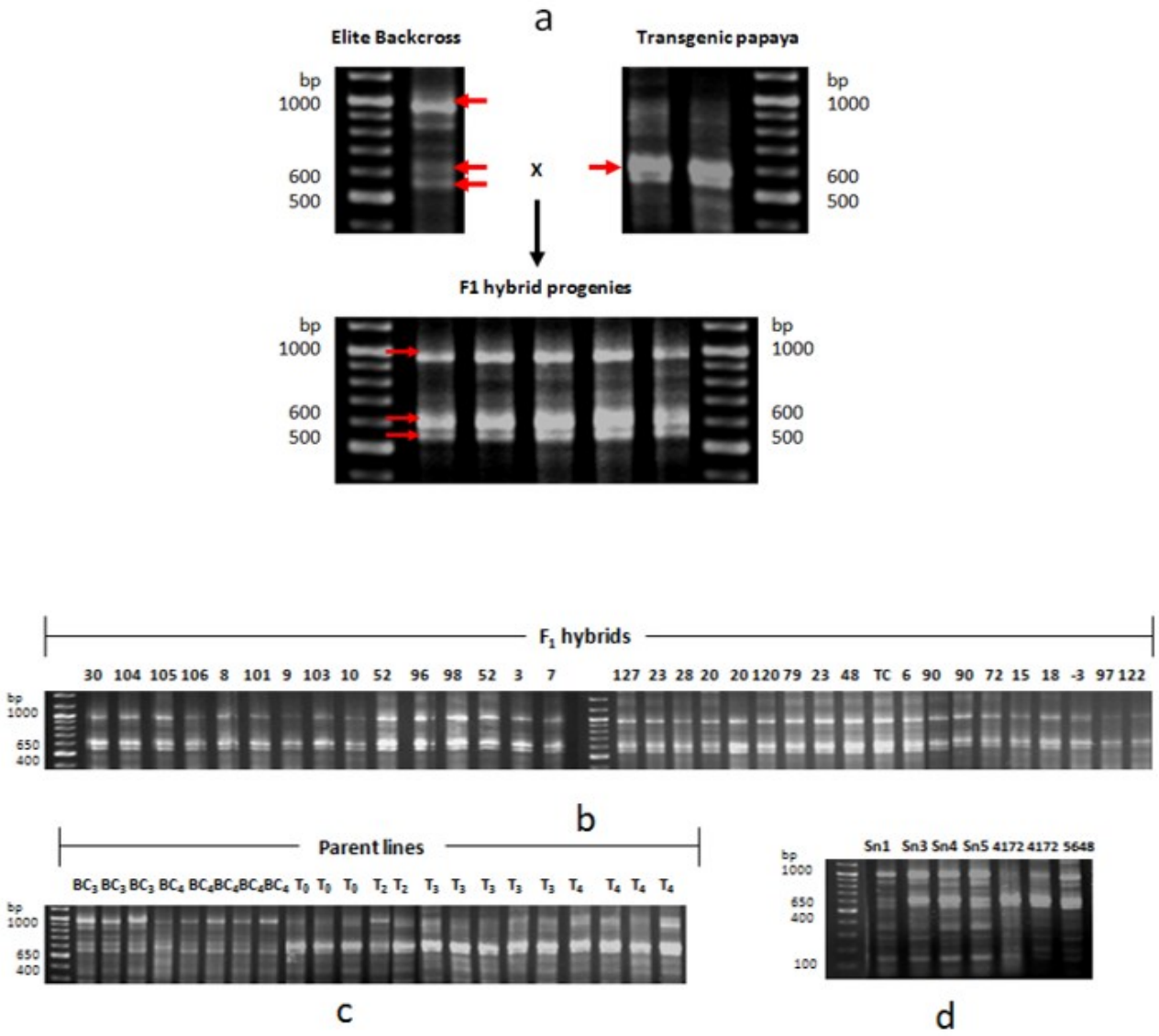

Fig. 3. DNA fingerprints of the hybrid papaya using CPY markers on (a) transgenic papaya and backcross, (b and c) 32 hybrid progenies, parent lines, and parent lines, BC_3 / BC_4 (♀) and transgenic papayas (♂), and (d) hybrid Sinta and its parents, 5648 (♀) and 4172 (♂).

the F_1 obtained its 500 bp and 250 bp from T4 and the 300 bp from BC3. CPMET did not give highly discriminating bands for the hybrid and the parentals. Thus, the markers CPY, BGAL, SSR 12 and SSR 35 showed amplification of specific and unique alleles among the parental lines, BC3 and T4 which provided hybridity bands in their crosses.

CONCLUSION

The increasing local production and commercialization of papaya underscores the importance of correct identification of varieties and assurance of genetic purity. This study showed that the identified microsatellite markers can effectively identify the papaya varieties and hybrids. The relatively high level of informativeness by the SSR markers indicates their capability to quantify genetic diversity and identify different genotypes of papaya. This molecular analysis will pave the way for more in-depth studies on the characterization of the papaya cultivars, accessions, and promising lines in the country, and contribute to breeding programs to develop new cultivars using the elite local cultivars.

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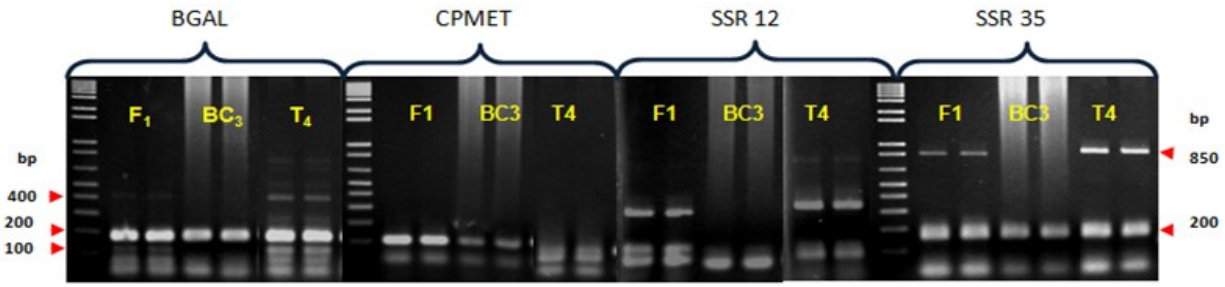

Fig. 4. Hybridity of polymorphic microsatellite markers between parental lines, backcross 3 (BC3), transgenic 4 (T_4), and papaya hybrids.

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