PHILIPP AGRIC SCIENTIST
Vol. 100 No. 1, 7–15
March 2017

ISSN 0031-7454

Genetic Diversity Analysis and DNA Fingerprinting of Pili (*Canarium ovatum* Engl.) using Microsatellite Markers

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Six microsatellite markers obtained from *Canarium album* and *Carica papaya* detected eight loci in the pili (*Canarium ovatum* Engl.) accessions with two markers, *Cas*C120 and SSR38, each amplifying two loci. A total of 43 alleles (5.38 alleles per locus) were detected in the 79 pili accessions assayed, while only 3.25 alleles per locus were obtained in *Canarium luzonicum* and in seven pili varieties registered with the National Seed Industry Council (NSIC). Polymorphism information content (PIC) values of the markers were relatively high across accessions (0.57) and varieties (0.45), indicating the ability of the markers to detect genetic diversity in the population assayed. Gene diversity was relatively high in the pili varieties (0.50) and in the 79 pili accessions (0.62) analyzed in the study, indicating recombination by cross pollination. Cluster analysis grouped the pili accessions and the seven varieties into two clusters. Group I was composed of accessions PDF65 and PDF20, while the other 77 accessions, including the seven pili varieties, clustered together forming Group II. Six of the varieties ('Katutubo', 'Lanuza', 'Magayon', 'Magnaye', 'Mayon I', and 'Orolfo') formed one sub-cluster under Group II, while 'Laysa' formed a different sub-cluster. Moreover, cluster analysis of the pili varieties and *C. luzonicum* showed the same trend wherein the six varieties formed one cluster, while 'Laysa' diverged from the group. DNA fingerprints of the pili accessions were generated from the banding patterns observed across the eight loci. The pili varieties gave unique DNA fingerprints, demonstrating the utility of the markers for varietal identification. This is the first report of a study in the Philippines on the molecular characterization of *C. ovatum* using DNA markers.

Key Words: pili, genetic diversity, DNA fingerprinting, microsatellites, SSRs

Abbreviations: NJ – neighbor joining, NSIC – National Seed Industry Council, PCR – polymerase chain reaction, PIC – polymorphism information content, SSRs – simple sequence repeats, OTUs – operational taxonomic units

INTRODUCTION

Canarium ovatum Engl., commonly known as pili, is a fruit tree indigenous to the Philippines and has its center of diversity in the Bicol region (Li 1970; Coronel 1994). There are about 75 *Canarium* species found in tropical Asia and the Pacific, nine of which are found in the Philippines but only two are of economic importance. Aside from *C. ovatum*, which is important for its edible nut, another species (*C. luzonicum*) is tapped for its oily resin commercially known as Manila elemi (Manalo and West 1940; Coronel 1994).

Pili is a dioecious plant species with a gametic chromosome number of n=23 (Villegas and Coronel 1980). Because of their dioecious nature and high degree of open pollination, pili nut trees grown from seeds have been reported to show great variation in many important horticultural characters (summarized by Coronel 1996). Therefore, varietal improvement has been mainly done by selection of superior trees. Wester (1929) selected the first pili tree, which was named 'Albay' after the province where it was collected. A more systematic method of evaluating and selecting superior pili accessions was done in 1946 in Los Baños, Laguna (Gonzalez and Bunoan 1947). Selected trees are asexually propagated and maintained in gene banks. One accession with outstanding fruit and yield qualities was a product of this selection and was later named

'Katutubo' (Coronel 1996). In 1994, the Fruit and Plantation Crops Varietal Improvement Group of the National Seed Industry Council (NSIC) established a set of varietal selection standards for the pili based on 'Katutubo' as reference variety (Coronel 1996). Currently, there are seven NSIC-registered varieties, namely, 'Katutubo', 'Lanuza', 'Laysa', 'Magayon', 'Magnaye', 'Mayon I' and 'Orolfo'.

Only two studies on genetic diversity of *C. ovatum* have been published both using isozymes. Sui et al. (1997) studied the polymorphisms of six isozyme systems (LAP, MDH, GPI, PGM, TPI and UGPP) across seven *Canarium* species, including *C. ovatum*. Mendioro et al. (2008) studied the genetic variability of two groups of *C. ovatum* accessions from the National Plant Genetic Resources Laboratory, Institute of Plant Breeding, Crop Science Cluster of the University of the Philippines Los Baños (UPLB) using 10 isozyme systems which detected a total of 18 presumptive loci. However, no investigation had been done before the present study on the genetic diversity or genotyping of *C. ovatum* using DNA markers.

Microsatellite or simple sequence repeat (SSR) markers are tandem repeats of DNA sequences of only a few base pairs (1–6 bp) in length which are found throughout the genome and are flanked by conserved sequences (Litt and Luty 1989). SSRs are highly polymorphic and co-dominant, and have been used widely for genetic analysis in crop improvement strategies (Sharapova et al. 2002). Some applications include diversity, pedigree, fingerprinting and genetic and quantitative trait loci mapping studies in various crops. Microsatellite markers have been utilized for diversity and fingerprinting studies in fruit trees including apple (Guilford et al. 1997), pear (Yamamoto et al. 2001), peach (fingerprinting and similarity, Hormaza 2002), papaya (Eustice et al. 2008; De Oliveira et al. 2010), mango (Azmat et al. 2016) and mandarin (Singh et al. 2016).

Microsatellites have been widely used and recognized as informative genetic markers, not only because of their high levels of polymorphism and high reproducibility, but also to a certain extent because of transferability of microsatellite loci across species. Transferability of microsatellites is a consequence of homologous conserved regions flanking the SSRs across taxonomic groups. Barbara et al. (2007) reported a comprehensive summary of cross-species marker success in plants, animals and fungi, which revealed high amplification success within and between genera in many plant and animal groups.

Although pili has been identified as one of seven major fruit trees in the Philippines, its potential as a world class product has not been realized. Therefore, it is important to study and conserve such a plant genetic resource. Molecular characterization of plant germplasm plays a vital role in the management and utilization of plant genetic resources (Karp 2002).

The objectives of this study were (1) to evaluate the genetic diversity and establish the DNA fingerprints of 79 pili accessions, including seven NSIC-registered varieties, using SSR markers from *Canarium album* and *Carica papaya*, and (2) to provide information on the evolutionary relationships and genetic diversity of *Canarium ovatum* and its relative *Canarium luzonicum*. This is the first report of a study in the Philippines on the molecular characterization of pili using DNA markers.

MATERIALS AND METHODS

Plant Materials

Genetic diversity analysis and DNA fingerprinting were performed on 79 *C. ovatum* accessions, including 7 NSIC-registered pili varieties ('Katutubo', 'Lanuza', 'Laysa', 'Magayon', 'Magnaye', 'Mayon I' and 'Orolfo') (Supplementary Table 1). Another *Canarium* species found in the Philippines, *C. luzonicum*, was also included in the study for reference and comparison of SSR profiles. Leaf samples were obtained from two germplasm collections maintained at the experimental orchards of UPLB, College, Laguna; from the scion grove of the Department of Agriculture Regional Field Unit V Pili Research and Technology Center (PRTC) in Tabaco, Albay, Camarines Sur; and from three private pili farms in San Rafael, Bulusan, Sorsogon in the Bicol region. The two UPLB pili collections are separately managed by the National Plant Genetics Resources Laboratory, Institute of Plant Breeding (IPB accessions) and the Fruit Crops Collection Orchard, Crop Science Cluster (Pili Drive accessions).

Table 1. Characteristics of the six simple sequence repeat (SSR) primers used for DNA fingerprinting of the NSIC-registered pili (*Canarium ovatum* Engl.) varieties.

Primer Name	Locus	Repeat Motif	GenBank Accession	Designed for	Reference	T_a (°C)
*Cas*A131	*Cas*A131	$(AG)_9$	FJ485697	*C. album*	Zhang et al. (2009)	50
*Cas*C183	*Cas*C183	$(CT)_9$	FJ485692	*C. album*	Zhang et al. (2009)	55
*Cas*C120	*Cas*C120	$(TC)_{22}$	FJ485689	*C. album*	Zhang et al. (2009)	50
SSR 12	P6K72CC	$(GA)_{14}^{imp}$	AC238636.1 AC238628.1	*C. papaya*	Cimagala (2011)	55
SSR 31	P6K108CC	$(ATTTT)_5^{p}$	AC238768.1 AC238636.1	*C. papaya*	Cimagala (2011)	50
SSR 38	P6K975CC	$(AAATTTGTACTTT)_4^{cmp}$	EF661024.1 GQ478573.2	*C. papaya*	Cimagala (2011)	53

DNA Isolation

Fresh leaves (2 g) were ground in liquid nitrogen to a fine powder using a mortar and pestle. Genomic DNA was isolated from the ground tissue according to a modified protocol (Doyle and Doyle 1990; Sharma et al. 2008). DNA concentration and quality were determined by spectrophotometry (SmartSpec™ 3000, Bio-Rad, USA). The concentrations of the genomic DNA were estimated from the absorbance at 260 nm and the quality was assessed by obtaining the ratio of A_{260} to A_{280}. The concentrations of the genomic DNA samples were normalized (100 ng µL^{-1}) and were run on 1% (w/v) agarose gel against lambda DNA standard (Roche Lifesciences, USA) to check the concentration and integrity prior to PCR.

SSR Markers and Polymerase Chain Reaction

A total of 24 SSR markers from *Canarium album* and *Carica papaya* were screened for polymorphism across the *C. ovatum* accessions. Six polymorphic markers, three from *C. album* and three from *C. papaya* (Table 1), were used for cross-amplification of SSRs in *C. ovatum* accessions and *C. luzonicum*. The annealing temperatures of the primers used were optimized for amplification of microsatellites in *C. ovatum*. PCR reactions were prepared in a total volume of 15 µL containing 10 mM Tris-HCl (pH 9.1), 50 mM KCl, 2 mM $MgCl_2$, 0.2 mM each dNTP, 0.5 µM each primer, 0.5 unit *Taq* DNA polymerase (Vivantis, Malaysia) and 100 ng template DNA. PCR amplifications were carried out as follows: initial denaturation at 94 °C for 5 min followed by 35 cycles of 94 °C for 1 min, optimized annealing temperature for each marker (Table 1) for 1 min, 72 °C for 1 min and a final denaturation at 72 °C for 5 min. Amplification products were resolved in 3 % (w/v) agarose gel in 1X TAE buffer, stained with 0.5 µg mL^{-1} ethidium bromide and viewed using a gel photo documentation system (GelDoc™, BioRad, USA).

Determination of DNA Fingerprints

The distinct and reproducible DNA fragments amplified were considered and scored. The size of each amplicon was estimated based on its migration on agarose gel relative to a DNA size ladder (1 Kb plus DNA ladder, Invitrogen) using the GelAnalyzer software. The banding patterns produced by the amplicons from each accession were designated with a number. The DNA fingerprint of each accession was assigned as the banding patterns observed for the loci *Cas*A131, *Cas*A183, *Cas*C120a, *Cas*C120b, SSR12, SSR31, SSR38a and SSR38b. A '0' was assigned to genotypes with null alleles or no amplification products for a particular SSR locus.

Data Analysis

Because of the haploid gametic chromosome number of pili, the allele scores were treated as haplotype data. The alleles amplified in the 8 SSR loci were converted into a numeric code, and a numeric matrix was developed by assigning values from 1 to the maximum number of alleles detected across the OTUs, prior to statistical analysis.

Polymorphism information content (PIC), a measure of the degree of variability at a locus, was computed using the following formula (Saal and Wricke 1999):

$$\text{PIC} = 1 - \sum_{i=1}^{l} p_i^2$$

where pi is the frequency of the ith allele out of the total number of alleles at an SSR locus and l is the total number of different alleles at that locus (Botstein et al. 1980).

Gene diversity, allele frequencies, shared allele genetic distance and bootstrap values (consensus of 1,000 trees) were calculated using the PowerMarker v3.25 software (Liu and Muse 2005). Genetic relationships were determined by cluster analysis using the neighbor joining (NJ) method, and

MEGA4 software (Tamura et al. 2007) was used to construct a dendrogram based on the similarity indices.

RESULTS AND DISCUSSION

Microsatellite markers were used to analyze the genetic diversity and to generate the DNA fingerprints of 79 *C. ovatum* accessions. The eight microsatellite loci analyzed revealed high genetic diversity in the pili accessions and generated unique DNA fingerprints for each of the NSIC-registered varieties; thus, they could be used for varietal identification.

Characteristics of SSR Markers

Microsatellites were successfully cross-amplified in the pili accessions analyzed and in *C. luzonicum* using the SSR markers from *C. album* and *C. papaya*. A total of 24 markers from *C. album* and *C. papaya* were screened and the six that produced polymorphic amplicons were used in the study. The transferability of the microsatellite markers from *C. album* may be explained by its 25 % genetic similarity with *C. ovatum* using isozymes as reported by Sui et al (1997). On the other hand, the papaya SSR primers used were designed from gene sequences obtained from the *C. papaya* draft genome (Cimagala 2011) and their success here indicates that genic microsatellites are transferable even among distantly related species (Varshney et al. 2005). Cross-species amplification of microsatellite loci have been successfully demonstrated in different plant species such as oak (Isagi and Suhandono 1997), peach (Cipriani et al. 1999), Canarium (Zhang et al. 2009) and passion fruit (Oliveira et al. 2013). In tropical trees, a high rate of transferability of microsatellite loci has been reported in Leguminosae (Dayanandan et al. 1997), Meliaceae (White and Powell 1997), Caryocaraceae (Collevatti et al. 1999) and among Eucalyptus species (Brondani et al. 1998; Zucchi et al. 2002).

Two markers, *Cas*C120 and SSR38, amplified two different loci designated *Cas*C120a, *Cas*C120b, SSR38a and SSR38b. This was also observed in the study of Cipriani et al. (2002) on microsatellite fingerprinting of olive (*Olea europaea* L.) cultivars, wherein five of the 30 microsatellite markers screened amplified two different loci. This may be the result of duplicated genes which are often found in eukaryotes. A total of 43 alleles, ranging from 4 (*Cas*A131, *Cas*A183, *Cas*C120b, SSR38b) to 10 (SSR12) alleles, were detected in the eight loci (5.38 alleles per loci) by the six polymorphic markers across the accessions studied.

Furthermore, no amplification products were obtained in some microsatellite loci and across several accessions assayed; thus, they were considered as null alleles. Null alleles are alleles at a microsatellite locus that are consistently not amplified to detected levels. Dakin and Avise (2004) reviewed the causes of microsatellite null alleles and these include (a) poor primer annealing due to nucleotide sequence divergence in the primers, particularly mutations in the 3′ end of the priming site, (b) amplification of size variant alleles, (c) PCR failure due to inconsistent DNA template quality and quantity, (d) Wahlund effect or inbreeding, and (e) sex linkage, wherein diploid species with digametic sex carries only one allele at any locus on the sex chromosome. Null alleles were observed across different pili accessions in all the loci assayed. The frequency of the null alleles observed in the microsatellite loci ranged from 0.05 (*Cas*A131) to 0.60 (SSR31). The presence of null alleles is common and has been observed in cross-species amplification of microsatellites in many plant species (cassava, Roa et al. 2000; hazelnut, Bassil et al. 2005; fig tree, Nazareno et al. 2009; Lecythidaceae, Guidugli et al. 2010; rubber tree, Mantello et al. 2012; Cactaceae, Moraes et al. 2014). Because pili is cross-pollinated, genetic recombination occurs during sexual reproduction, which may lead to the loss or modifications in the primer annealing sites and may be one of the causes of the null alleles detected. Another possible reason for the presence of null alleles is sex linkage since pili is a dioecious species. Random association of genes from the gametes of the male and female parents during recombination may also explain the presence of null alleles in some of the pili accessions studied.

PIC values, which were calculated to measure the degree of informativeness of the loci detected by the SSR markers, ranged from 0.41 (*Cas*A131) to 0.73 (SSR12) with an average of 0.57 (Table 2), indicating that the loci were moderate to highly informative and could therefore detect and quantify genetic diversity in the population studied. The average gene diversity was also high at 0.62 (ranging from 0.48 to 0.77), indicating high genetic variability

Table 2. Amplicon size range, number of alleles (N_A), gene diversity and polymorphism information content (PIC) of the eight simple sequence repeat (SSR) loci studied across the 79 pili (*Canarium ovatum* Engl.) accessions and *C. luzonicum*.

Locus	Size Range (bp)	N_A	Gene Diversity	PIC
*Cas*A131	200–300	4	0.48	0.41
*Cas*A183	190–220	4	0.66	0.60
*Cas*C120a	210–278	5	0.68	0.64
*Cas*C120b	100–134	4	0.61	0.57
SSR12	100–263	10	0.77	0.73
SSR31	200–270	6	0.56	0.49
SSR38a	198–239	4	0.48	0.44
SSR38b	90–142	6	0.74	0.70
Average		**5.38**	**0.62**	**0.57**

among the *Canarium* accessions studied (Table 2). This is consistent with the report of Mendioro et al. (2008) on the genetic diversity of 30 pili accessions using three isozymes with nine presumptive loci (EST, ACP and ALP) where relatively high gene diversity (average H_e = 0.475) was also observed. High genetic diversity is expected in the pili because it is a dioecious and obligate cross-pollinating crop. Genetic variability can be brought about by recombination during sexual reproduction.

Genetic Analysis of Pili Accessions

Cluster analysis was performed using the neighbor joining (NJ) method based on shared allele genetic distance (Supplementary Table 2). NJ tree rooted to the outgroup *C. luzonicum* was constructed to illustrate the genetic relationships (Fig. 1). NJ analysis clustered the pili accessions into two groups. The first group is composed of accessions PDF65 and PDF20 while the 77 accessions, including the seven varieties, formed the second major group. Group I is divergent from the rest of the population and is almost as divergent as *C. luzonicum*. 'Laysa' clustered with accession PDF86 while the other six varieties 'Mayon I', 'Lanuza', 'Katutubo', 'Orolfo', 'Magayon' and 'Magnaye' formed one cluster in Group II, indicating that they are more closely related. Varietal improvement in pili is usually accomplished by evaluation and selection of superior trees maintained in germplasm collection, which could possibly explain the clustering of the pili varieties. Moreover, no association between genotype and origin of collection can be established by the cluster analysis because the pili accessions in the germplasm maintained at UPLB were collected from the Bicol region (mostly from Oas, Albay). Similar results had

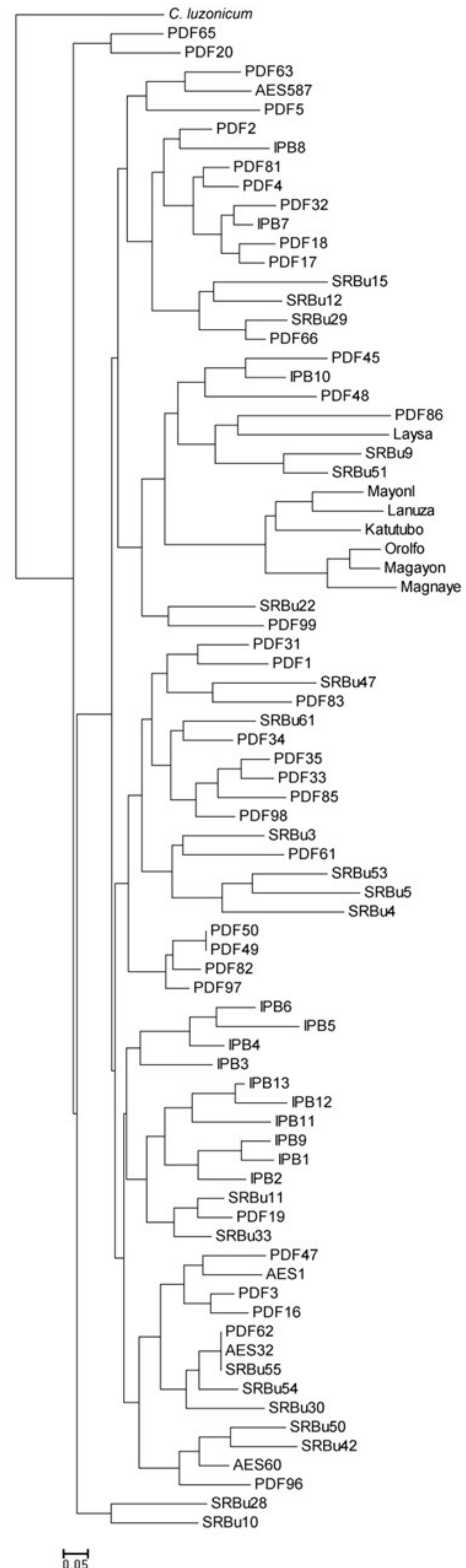

Fig. 1. Rooted neighbor joining (NJ) tree based on shared allele distance of the pili (*Canarium ovatum* Engl.) accessions studied. Accession codes: IPB - Institute of Plant Breeding, PDF - Pili Drive, SrBu - San Rafael, Bulusan, AES - DA PRTC Albay Experiment Station

previously been obtained by Mendioro et al. (2008) in their cluster analysis of 30 pili accessions collected from the same region. Furthermore, asexual propagation of pili by grafting or budding is practiced in nurseries, thus clonal seedlings may be transported and introduced in any part of the Bicol region or the country.

As presented in Supplementary Table 2, the shared allele genetic distances of the *Canarium* species studied had values ranging from 0.00 (100 % of alleles shared) to 1.00 (no shared alleles). Pairwise comparison of the shared allele distances showed that PDF62/AES32, SRBu55/AES32, PDF62/SRBu55 and PDF49/PDF50 shared the same set of alleles. On the other hand, 175 pairs of individuals had no common alleles, indicating high variability in the population based on the 43 alleles detected.

Genetic Diversity and Relationships of Pili Varieties

The genetic variability among the seven pili varieties was also evaluated using the cross-amplified SSR markers from *C. album* and papaya. The eight loci analyzed detected a range of two (SSR31 and SSR38a) to seven (SSR12) alleles across the seven pili varieties and *C. luzonicum* with an average of 3.25 alleles per locus (Table 3). The low number of alleles detected per locus could be explained by the narrow genetic base analyzed consisting of the seven pili varieties. However, the low number of alleles detected does not necessarily affect the ability of the markers to discriminate between genotypes. In a similar study on cotton (Islam et al. 2012), three microsatellite markers that were assayed across eight cultivars detected eight alleles (2.64 alleles per marker) which still enabled the differentiation of the cotton varieties. The average gene diversity was 0.50, which is still relatively high in spite of the small population size studied. The PIC values obtained ranged from 0.19 (SSR31 and SSR38a) to 0.82 (SSR12) with an average of 0.45, which indicate that the loci are informative and could therefore discriminate among the individuals in the population studied (Islam et al. 2012).

Cluster analysis was also performed using the rooted NJ method to illustrate genetic relationships of pili varieties and *C. luzonicum* (Fig. 2). Bootstrap analysis of 1000 trees was also done to support cluster analysis and significant bootstrap values (>50) are indicated in the dendrogram. The rooted NJ tree clustered together all the pili varieties except ‘Laysa’ which diverged from the group. ‘Magnaye’, ‘Orolfo’ and ‘Magayon’ are the most closely evolutionary related among the pili varieties while ‘Katutubo’, ‘Lanuza’ and ‘Mayon I’ complete the group; however, there is no bootstrap support to distinguish which of these three varieties is more closely related to the subgroup formed by ‘Magnaye’, ‘Orolfo’ and ‘Magayon’. On the other hand, ‘Laysa’ is divergent from the rest of the *C. ovatum* genotypes, which was consistent with the phylogenetic analysis of the pili accessions. Moreover, the genotypes which tend to cluster together also showed similar DNA fingerprints.

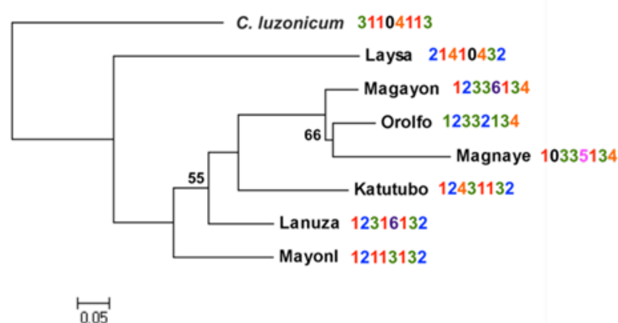

Fig. 2. Rooted neighbor joining (NJ) tree based on shared allele distance of NSIC-registered pili (*Canarium ovatum* Engl.) varieties. Significant bootstrap values are shown and the unique DNA fingerprints are adjacent to accession names.

Table 3. Number of alleles (N_A), gene diversity and polymorphism information content (PIC) of the eight simple sequence repeat (SSR) loci studied across the seven pili (*Canarium ovatum* Engl.) varieties and *C. luzonicum*.

Locus	N_A	Gene Diversity	PIC
*Cas*A131	3	0.41	0.37
*Cas*A183	3	0.53	0.47
*Cas*C120a	3	0.62	0.55
*Cas*C120b	3	0.59	0.51
SSR12	7	0.84	0.82
SSR31	2	0.22	0.19
SSR38a	2	0.22	0.19
SSR38b	3	0.59	0.51
Average	**3.25**	**0.50**	**0.45**

DNA Fingerprinting

The banding patterns for each of the detected loci across the genotypes assayed were determined and assigned with a digit (i.e. 1, 2,…), and loci which had no amplification products (null alleles) were assigned with a ‘0’. Each genotype was then assigned with a fingerprint, a number wherein each

digit corresponds to the observed banding pattern for loci *Cas*A131, *Cas*A183, *Cas*C120a, *Cas*C120b, SSR12, SSR31, SSR38a and SSR38b, respectively. The DNA fingerprints of all the accessions assayed are summarized in Supplementary Table 1. Only a few accessions gave identical fingerprints, particularly PDF49 and PDF50 (11201004), and PDF62, SRBu55 and AES32 (21200000). Pairwise comparison of the shared allele genetic distances of these accessions also indicate that they share the same set of alleles detected by the microsatellite markers (Supplementary Table 2). The fingerprint of SRBu54 (22200000) differs only at locus *Cas*A183 from the fingerprints of PDF62, SRBu55 and AES32. Other accessions with DNA fingerprints that differ at a single locus are IPB12 and IPB13 (at locus SSR38a), PDF3 and PDF16 (at *Cas*A183), PDF17 and PDF18 (at SSR12) and PDF4 and PDF66 (at SSR38b). The high variation observed in the DNA fingerprints across the pili accessions and varieties is consistent with the high gene diversity detected by the microsatellite markers analyzed.

The seven NSIC-registered pili varieties as well as *C. luzonicum* gave unique sets of fingerprints: 'Katutubo' - 12431132, 'Lanuza' - 12316132, 'Laysa' - 21410432, 'Magayon' - 12336134, 'Magnaye' - 10335134, 'Mayon I' - 12113132, 'Orolfo' - 12332134 and *C. luzonicum* - 31104113 (Fig. 3). Moreover, the DNA fingerprints support the cluster analysis of the pili varieties where genotypes with similar fingerprints tend to cluster together.

CONCLUSION

Cross-species amplification of microsatellite loci in *C. ovatum* was able to assess the genetic diversity of the *C. ovatum* accessions and was able to discriminate among the seven NSIC-registered varieties. The cost of development of SSR markers is high and cross-species amplification of microsatellites is a cost-effective means of assessing the genetic diversity of a neglected crop such as pili with very limited DNA sequence information available.

The findings of the study demonstrated that *C. ovatum* has high genetic variability. It also provided insight into the evolutionary relationships of *C. ovatum* and *C. album* because of the cross transferability of the microsatellite markers. Furthermore, the study contributed to the molecular characterization of existing pili germplasms, including the NSIC-registered varieties, which is important in the management and utilization of plant genetic resources.

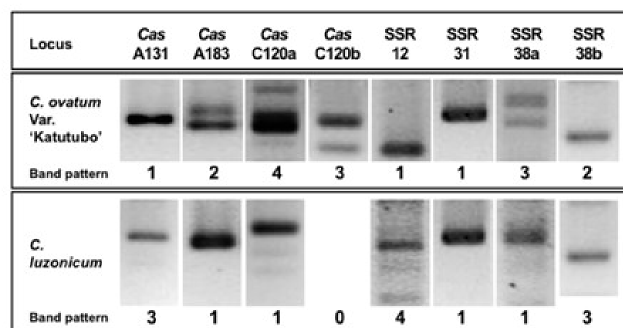

Fig. 3. DNA fingerprint of *C. ovatum* var. 'Katutubo' and *C. luzonicum* showing the amplification products across the eight microsatellite loci and the corresponding band pattern number

NOTE: Supplementary Tables 1 and 2 are available from the authors upon request.

REFERENCES CITED

AZMAT MA, KHAN AA, KHAN IA, RAJWANA IA, CHEEMA HMM, KHAN AS. 2016. Morphological characterization and SSR based DNA fingerprinting of elite commercial mango cultivars. Pak J Agric Sci 53 (2): 321–330.

BARBARA T, PALMA-SILVA C, PAGGI GM, BERED F, FAY MF, LEXER C. 2007. Cross-species transfer of nuclear microsatellite markers: potential and limitations. Mol Ecol 16: 3759–3767.

BASSIL NV, BOTTA R, MEHLENBACHER SA. 2005. Microsatellite markers in hazelnut: Isolation, characterization, and cross-species amplification. J Am Soc Hort Sci 130(4): 543–549.

BOTSTEIN D, WHITE RL, SKOLNICK M, DAVIS R. 1980. Construction of a genetic linkage map in man using restriction fragment polymorphisms. Am J Hum Genet 32: 314–331.

BRONDANI RPV, BRONDANI C, TARCHINI R, GRATTA-PAGLIA D. 1998. Development, characterization and mapping of microsatellite markers in *Eucalyptus grandis* and Europhylla. Theor Appl Genet 97: 816–827.

CIMAGALA KC. 2011. Development and characterization of SSR markers for fingerprinting, hybridity testing and sex determination of transgenic papaya with long shelf life and its PRSV resistant hybrid [MS thesis].

College, Laguna, Philippines: University of the Philippines Los Baños. 71 p. (Available at the UPLB Library).

CIPRIANI G, LOT G, HUANG WG, MARRAZO M, PETERLUNGER E, TESTOLIN R. 1999. AC/GT and AG/CT microsatellite repeats in peach [*Prunus persica* (L.) Batsch]: isolation, characterisation and cross-species amplification in Prunus. Theor Appl Genet 99: 65–72.

CIPRIANI G, MARRAZZO MT, MARCONI R, CIMATO A, TESTOLIN R. 2002. Microsatellite markers isolated in olive (*Olea europaea* L.) are suitable for individual fingerprinting and reveal polymorphism within ancient cultivars. Theor Appl Genet 104: 223–228.

COLLEVATTI RG, BRONDANI RV, GRATTAPAGLIA D. 1999. Development and characterization of microsatellite markers for genetic analysis of a Brazilian endangered tree species *Caryocar brasiliense*. Heredity 83: 748–756.

CORONEL RE. 1994. History and Current Status of Pili Nut (*Canarium ovatum*): Production in the Philippines. In: Stevens ML et al., editors. South Pacific Indigenous Nuts; 1994 October 31–November 4; Port Vila, Vanuatu: Australian Centre for International Agricultural Research. p. 134–139.

CORONEL RE. 1996. Pili Nut. *Canarium ovatum* Engl. Promoting the Conservation and Use of Underutilized and Neglected Crops. 6. Rome, Italy: Institute of Plant Genetics and Crop Plant Research, Gatersleben/ International Plant Genetic Resources Institute. 55 p.

DAKIN EE, AVISE JC. 2004. Microsatellite null alleles in parentage analysis. Heredity 93: 504–509.

DAYANANDAN S, BAWA KS, KESSELI R. 1997. Conservation of microsatellites among tropical trees (Leguminosae). Am J Bot 84: 1658–1663.

DE OLIVEIRA EJ, AMORIN VBO, MATOS ELS, COSTA JL, CASTELLEN MDS, PADUA JG, DANTAS JLL. 2010. Polymorphism of microsatellite markers in papaya (*Carica papaya* L.). Plant Mol Biol Rep 28: 519–530.

DOYLE JJ, DOYLE JL. 1990. Isolation of plant DNA from fresh tissue. Focus 12: 13–15.

EUSTICE M, YU Q, LAI CW, HOU S, THIMMAPURAM J, LIU L, ALAM M, MOORE PH, PRESTING GG, MING R. 2008. Development and application of microsatellite markers for genomic analysis of papaya. Tree Genet Genomes 4: 333–341.

GONZALEZ LG, BUNOAN JC. 1947. Variability of pili trees grown in the College of Agriculture. Philipp Agric 31(1): 60–65.

GUIDUGLI MC, ACCORONI KAG, MESTRINER MA, CONTEL, EPB, MARTINEZ CA, ALZATE-MARIN AL. 2010. Genetic characterization of 12 heterologous microsatellite markers for the giant tropical tree *Cariniana legalis* (Lecythidaceae). Genet Mol Biol 33(1): 131–134.

GUILFORD P, PRAKASH S, ZHIU JM, RIKKERINK E, GARDINER S, BASSETT H, FORSTER R. 1997. Microsatellites in *Malus domestica* (apple): abundance, polymorphism and cultivar identification. Theor Appl Genet 94: 249–254.

HORMAZA JI. 2002. Molecular characterization and similarity relationships among apricot (*Prunus armeniaca* L.) genotypes using simple sequence repeats. Theor Appl Genet 104: 321–328.

ISAGI Y, SUHANDONO S. 1997. PCR primers amplifying microsatellite loci of *Quercus myrsinifolia* Blume and their conservation between oak species. Mol Ecol 6: 897–899.

ISLAM MN, MOLLA MR, ROHMAN MM, HASANUZZAMAN M, ISLAM SMN, RAHMAN L. 2012. DNA fingerprinting and genotyping of cotton varieties using SSR markers. Not Bot Horti Agrobo 40 (2): 261–265.

KARP A. 2002. The new genetic era: will it help us in managing genetic diversity? In: Engels JMM, Rao VR, Brown AHD, Jackson MT, editors. Managing Plant Genetic Diversity. Rome, Italy: International Plant Genetic Resources Institute. p. 43–56.

LI HL. 1970. The origin of cultivated plants in Southeast Asia. Econ Bot 24(1): 3–19.

LITT M, LUTY JA. 1989. A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. Am J Hum Genet 44: 397–401.

LIU K, MUSE SV. 2005. PowerMarker: Integrated analysis environment for genetic marker data. Bioinformatics 21(9): 2128–2129.

MANALO GD, WEST AP. 1940. Analysis and composition of Manila elemi. Philipp J Sci 78(1): 111–120.

MANTELLO CC, SUZUKI FI, SOUZA LM, GONCALVES PS, SOUZA AP. 2012. Microsatellite marker development for the rubber tree (*Hevea brasiliensis*): characterization and cross-amplification in wild *Hevea* species. BMC Res Notes 5: 329.

MENDIORO MS, DIAZ MGQ, VELASCO VME, ALCARAZ MC, LALAMUNAN RC, AMOLOZA KG, VILLAMAEL LN. 2008. Genetic characterization of pili (*Canarium ovatum* Engl.) from Albay, Camarines

Norte, and Camarines Sur through isozyme analysis. Phil J Sci 137(2): 115–125.

MORAES EM, CIDADE FW, SILVA GAR, MACHADO MC. 2014. Polymorphic microsatellite markers for the rare and endangered cactus *Uebelmannia pectinifera* (Cactaceae) and its congeneric species. Genet Mol Res 13(4): 10359–10366.

NAZARENO AC, PEREIRA RAS, FERES JM, MESTRINER MA, ALZATE-MARIN AL. 2009. Transferability and characterization of microsatellite markers in two neotropical *Ficus* species. Genet Mol Biol 32(3): 568–571.

OLIVEIRA GAF, PÁDUA JG, COSTA JL, DE JESUS ON, DE CARVALHO FM, DE OLIVEIRA EJ. 2013. Cross-species amplification of microsatellite loci developed for *Passiflora edulis* Sims. in related *Passiflora* species. Braz Arch Biol Tech 56(5): 785–792.

ROA AC, CHAVARRIAGA-AGUIRRE P, DUQUE MC, MAYA MM, BONIERBALE MW, IGLESIAS C, TOHME J. 2000. Cross-species amplification of cassava (*Manihot esculenta*) (Euphorbiaceae) microsatellites: allelic polymorphism and degree of relationship. Am J Bot 87: 1647–1655.

SAAL B, WRICKE G. 1999. Development of simple sequence repeat markers in rye (*Secale cereale* L.). Genome 42: 964–972.

SHARAPOVA N, McMULLEN MD, SCHULTZ L, SCHROEDER S, SANCHEZ-VILLEDA H, GARDINER J, BERGSTROM D, HOUCHINS K, MELIA-HANCOCK S, MUSKET T, DURU N, POLACCO M, EDWARDS K, RUFF T, REGISTER JC, BROUWER C, THOMSON R, VELASCO R, CHIN E, LEE M, WOODMANPCLIKEMAN W, LONG MJ, LISCUM E, CONE K, DAVIS G, COE HE. 2002. Development and mapping of SSR markers for maize. Plant Mol Biol 48: 463–481.

SHARMA K, AK MISHRA, MISRA RS. 2008. A simple and efficient method for extraction of genomic DNA from tropical tuber crops. Afr J Biotechnol 7(8): 1018–1022.

SINGH G, AULAKH PS, SARAO NK, SIDHU GS, RATTANPAL HS. 2016. Genetic diversity and DNA fingerprinting of indigenous and exotic mandarin genotypes in India using SSR markers. Aust J Crop Sci 10(1): 24–31.

SUI L, ZEE F, MANSHARDT RM, ARADHYA MK. 1997. Enzyme polymorphisms in *Canarium*. Sci Hort 68: 197–206.

TAMURA K, DUDLEY J, NEI M, KUMAR S. 2007. *MEGA4*: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol 24: 1596–1599.

VARSHNEY RK, GRANER A, SORRELIS ME. 2005. Genic microsatellite markers in plants: features and applications. Trends Biotechnol 23(1): 48–55.

VILLEGAS VN, CORONEL RE. 1980. Cytology of pili and barobo. Philipp Agric 63: 174–178.

WESTER PJ. 1929. Observations on agriculture in the Philippines. Philipp Agric Rev 5(3): 365–370.

WHITE G, POWELL W. 1997. Cross-species amplification of SSR loci in the Meliaceae family. Mol Ecol 6: 1195–1197.

YAMAMOTO T, KIMURA Y, SAWAMURA Y, KOTOBUKI K, BAN Y, HAYASHI T, MATSUTA N. 2001. SSRs isolated from apple can identify polymorphism and genetic diversity in pear. Theor Appl Genet 102: 865–870.

ZHANG X, YE WH, CAO HL, WANG ZF, SHEN H, LIAN JY. 2009. Isolation and characterization of microsatellites in Chinese white olive (*Canarium album*) and cross-species amplification in *Canarium pimela*. Conserv Genet 10: 1833–1835.

ZUCCHI M, BRONDANI R, PINHEIRO J, BRONDANI C, VENCOVSKY R. 2002. Transferability of microsatellite markers from *Eucalyptus* spp. to *Eugenia dysenterica* (Myrtaceae family). Mol Ecol Notes 2: 512–513.