J.Natn.Sci.Foundation Sri Lanka 2017 45 (3): 255- 264
DOI: http://dx.doi.org/10.4038/jnsfsr.v45i3.8190

RESEARCH ARTICLE

Morphological, SSR and ISSR marker based genetic diversity assessment of mountain papaya germplasm in comparison with *Carica papaya*

W.A.D.L.R. Warnakula[1], N.S. Kottearachchi[1*] and K. Yakandawala[2]

[1] *Department of Biotechnology, Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka, Makandura, Gonawila (NWP).*

[2] *Department of Horticulture and Landscape Gardening, Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka, Makandura, Gonawila (NWP).*

Revised: 02 October 2016; Accepted: 16 February 2017

Abstract: The genetic diversity in papaya cultivars is essentially important as it provides the basis for varietal improvement. In this regard a study was initiated to assess the genetic diversity of the commercial *Carica papaya* cultivars in Sri Lanka and to introduce mountain papaya (*Vasconcellea cundinamarcensis*), which is reported to contain cold resistance and papaya ring spot virus (PRSV-P) resistant trait. Twenty one accessions of *Carica papaya* and mountain papaya were assessed by morphological, simple sequence repeat (SSR) and inter simple sequence repeat (ISSR) markers. A total of 33 alleles were generated with an average frequency of 2.5 alleles per marker from 11 SSR and 2 ISSR markers. Morphological and molecular marker based cluster analyses revealed that there was no clear distinction among the *C. papaya* cultivars grown in different geographical areas in Sri Lanka, while mountain papaya was highly distinct from the other *C. papaya* accessions. Except three SSR markers, all the other markers were polymorphic between mountain papaya and *C. papaya* accessions. Of the 33 alleles produced, 12 alleles were common for both *C. papaya* and mountain papaya indicating the potential relatedness to *C. papaya*. This investigation revealed both the genetic diversity and the relatedness of mountain papaya with *C. papaya* so as to use it as a potential source for the improvement of *C. papaya* by hybridisation.

Keywords: *Carica papaya*, ISSR markers, morphological characterisation, mountain papaya, SSR markers.

INTRODUCTION

Most of the *Caricaceae* species are considered as unexploited species except for *Carica papaya* L., which is commercially grown in many parts of the world. Papaya is a good source of vitamins and it is widely grown for consumption as a fresh fruit and for use in drinks, jams, candies, etc. Papaya also has pharmaceutical and industrial values due to its proteins and alkaloids. Of these, papain is the most important industrially valuable proteolytic enzyme that is produced in the milky latex of green, unripe papaya fruits (Moussaoui *et al.*, 2001). In Sri Lanka there are several *C. papaya* cultivars including introduced varieties from other countries, hybrid varieties and local-traditional cultivars. Currently 7,108 ha are under cultivation and the annual production is 84,606 mt (DOA, 2014).

The family *Caricaceae* comprises five genera and about 34 − 35 species (Carvalho *et al.,* 2015). Previously, the genus *Carica* contained 21 species, and following the recommendation of Badillo (2000) *Carica* was split into two genera creating an additional genus called *Vasconcellea* comprising 20 wild species. Presently the genus *Carica* contains only one species, *Carica papaya*, which is the most economically valuable *Caricaceae* species worldwide.

The mountain papaya plant has been studied by many scientists in the world due to its economically important traits such as the potential for cold tolerance (Muthulakshmi *et al.*, 2007), papaya ring spot virus (PRSV-P) resistance (Dillon *et al*., 2006), higher sugar content of the fruit (Drew *et al.*,1998), and genetic relatedness to *C. papaya* (O'Brien & Drew, 2010). As the closest relative of *C. papaya* species, mountain

* Corresponding author (kottearachchins@yahoo.com)

papaya has the likelihood to hybridise and exchange genes, which are associated with economically important traits (Badillo, 2000). Both *Carica* and *Vasconcellea* are genetically diploids and share the same chromosome number, 2n = 18 (Storey, 1976). Several research studies have indicated that pollination of *C. papaya* with pollen from wild papayas (and *vice versa*) successfully facilitate the fertilisation (Manshardt & Wenslaff, 1989; Drew *et al*., 1998).

Mountain papaya existed in the montane zone of Sri Lanka has been recognised as *Carica pubescence* in the Revised Handbook to the Flora of Ceylon (Dasanayake, 1995) and in the Check List of the Flowering Plants of Sri Lanka (Senaratna, 2001). It has been reported in the Halgolla Estate, which is located in the southeastern edge of the Kegalle District and close to the western boundary of the Nuwara Eliya District (Weerakoon *et al*., 2009) as an uncultivated papaya species. Mountain papaya is a sparsely branched herbaceous tree, habituated in the highest elevation level, in Shanthipura, Nuwara Eliya. Taxonomy of the mountain papaya accession in Sri Lanka has not been explained clearly and naturally hybridised genotypes have not been reported in Sri Lanka.

In Sri Lanka, there are four main types of commercial papaya: Rathne, Sinta, Red Lady and local/ traditional varieties, and their genetic relatedness has not been studied at molecular level using simple sequence repeat (SSR) and inter simple sequence repeat (ISSR) markers. Allelic variation of the economically important traits needs to be utilised in breeding programmes and they can be revealed by SSR and ISSR marker based assessments. Although mountain papaya naturally possesses a number of desirable traits, its proper characterisation has not been conducted and neglected as it grows in the wild. Therefore, this research study was aimed at assessing the genetic diversity of the commercial papaya accession in Sri Lanka along with mountain papaya, and to explore its potential traits to improve *C. papaya* accessions in the country.

METHODOLOGY

Plant materials

Leaf samples were collected from 18 genotypes of *C. papaya* distributed in the Central, North Western and Western provinces of Sri Lanka and three mountain papaya samples were collected from Shanthipura,

Table 1: Details of the papaya germplasm and their locations

Sample no.	Germplasm	Code	Source	District
1	Red Lady	RDL01	Katugasthota	Kandy
2	Local	LCL01	Katugasthota	Kandy
3	Sinta	SIN01	PVIC, Homagama	Colombo
4	Breeders line	BRL01	PVIC, Homagama	Colombo
5	Breeders line	BRL02	PVIC, Homagama	Colombo
6	Local	LCL02	PVIC, Homagama	Colombo
7	Local	LCL03	SCS, Batalegoda	Kurunegala
8	Sinta	SIN02	HRF, Walpita	Gampaha
9	Red Lady	RDL02	HRF, Walpita	Gampaha
10	Rathna	RTN01	HRF, Walpita	Gampaha
11	Local	LCL04	Bopitiya	Kurunegala
12	Red Lady	RDL03	RARDC, Makandura	Kurunegala
13	Sinta	SIN03	RARDC, Makandura	Kurunegala
14	Local	LCL05	Dikkele	Kurunegala
15	Mountain papaya	MTP01	Shanthipura	Nuwara Eliya
16	Mountain papaya	MTP02	Shanthipura	Nuwara Eliya
17	Mountain papaya	MTP03	Shanthipura	Nuwara Eliya
18	Local	LCL06	Katukithula, Ramboda	Kandy
19	Local	LCL07	Kuliyapitiya	Kurunegala
20	Local	LCL08	Giriulla	Kurunegala
21	Local	LCL09	Bopitiya	Kurunegala

PVIC - Plant Virus Indexing Centre; SCS - Seed Certification Station; HRF - Horticultural Research Farm; RARDC - Regional Agricultural Research and Development Centre

Nuwara Eliya, Sri Lanka for DNA extraction. The local or common names ascribed to the individual accessions were recorded as a reference to the material collected, and each accession was assigned a taxon code based on their local name (Table 1).

Morphological analysis

A total of 14 vegetative and reproductive characters were observed *in situ* from the selected papaya accessions (Table 2). A score was assigned (Table 3) to each morphological character following the papaya descriptor list published by the International Board for Plant Genetic Resources (IBPGR, 1988). As the samples were collected from different geographical areas in the country, only the qualitative traits were accounted in this study following the study conducted by Madarbokus and Ranghoo-Sanmukhiya (2012). The sex of each variety was determined by the floral morphology (Jiménez *et al.*, 2012). Visual morphological characters of the plant specimens of mountain papaya were further clarified by examining digital images of herbarium specimens in the Kew Herbarium database (*http://www.kew.org/science*).

Table 2: Qualitative traits and their respective score

No.	Qualitative trait	Scores
1	Stem type	1 - Single, 2 - Branched
2	Stem colour	1 - Light grey, 2 - Greyish brown
3	Stem pigmentation	1 - Mostly upper, 2 - Indiscriminate
4	Colour of mature leaf petiole	1 - Pale green, 2 - Dark green, 3 - Red purple, 4 - Green purple
5	Waxiness on leaf surface	1 - Present, 2 - Absent
6	Leaf segments (lobes)	1 - Nine segments, 2 - Seven segments
7	Type of flowering	1 - Solitary, 2 - Inflorescence
8	Colour of inflorescence stalk	1 - Cream, 2 - Yellow purple, 3 - Greenish
9	Colour of flower	1 - Greenish, 2 - Dark green
10	Fruit shape	1 - Oblong, 2 - Reniform, 3 - Oval, 4 - Elongate, 5 - Club, 6 - Acron, 7 - Globular
11	Fruit skin colour	1 - Deep yellow, 2 - Yellow, 3 - Orange
12	Fruit flesh colour	1 - Bright yellow, 2 - Orange, 3 - Reddish orange, 4 - Light yellow
13	Seed colour	1 - Black, 2 - Brown black, 3 - Tan
14	Flesh aroma	1 - Mild, 2 - Moderate, 3 - Strong

Genomic DNA extraction

A healthy tender leaf was used for extracting DNA. Fresh leaf tissues (200 mg) were ground in 600 µL of cetyl trimethyl ammonium bromide (CTAB) extraction buffer [2 % CTAB, 5 M NaCl, 0.5 M ethylene diamine tetra acetic acid (EDTA) at pH 8, Tris-HCL (trizma base hydrochloric acid) at pH 8 and 2 % ß-mercaptoethanol] using a mortar and pestle (Doyle & Doyle, 1987). The extract was transferred to a clean eppendorf tube and then incubated in a water bath at 60 °C for 30 min with occasional swirling. After incubating, the mixture was centrifuged at 12,000 rpm for 5 min in a microcentrifuge (Prism C2500, Labnet, NJ, USA) and the supernatant was transferred to a clean eppendorf tube. Two-thirds volume of chloroform: isoamyl alcohol (24:1, v/v) was added to the tube, and the tubes were centrifuged at 10,000 rpm for 10 min. The aqueous phase was transferred to a new tube and DNA was precipitated by the addition of two-thirds volume of ice-cold iso propanol. The tube was incubated at -20 °C for 30 min and centrifuged at 12,000 rpm for 10 min. The precipitated DNA was washed with 70 % ice-cold ethanol by centrifugation at 15,000 rpm for another 10 min. The DNA pellets were then dried and re-suspended in 300 µL of TE buffer and stored at -20 °C before using for SSR and ISSR analyses.

PCR assay

PCR amplification of the extracted DNA was conducted with 11 SSR primers (Ramos *et al*., 2011), and 2 ISSR (da Costa *et al.*, 2011) primers in a thermal cycler (Mycycler, BioRAD, CA, USA). The annealing temperatures (T_A) were optimised for each primer (Table 4). PCR amplification was carried out in 10 µL volumes of PCR mixture. Each reaction mixture contained 100 ng of genomic DNA, 1.6 µM of each primer, 1.2 x PCR buffer, 0.3 µM of dNTP, 0.075 U *Taq* DNA polymerase (Dream Taq, Fermentas) and PCR-grade water. The reaction mix was preheated at 95 ºC for 5 min followed by 35 cycles of 1 min denaturation at 95 ºC, 30 s annealing at optimised temperature and extension at 72 ºC for 1 min with the

Table 3: Variation in morphological characters among papaya germplasm

Sample number	Code	Qualitative character													
		Stem type	Stem colour	Stem pigmentation	Leaf petiole colour	Leaf segments	Leaf waxiness	Flower type	Flower colour	Flower stalk colour	Fruit shape	Ripen fruit skin colour	Flesh colour	Seed colour	Flesh aroma
1	RDL01	1	2	1	1	1	1	1	1	1	7	1	3	1	2
2	LCL01	1	2	2	2	1	1	2	1	1	4	2	1	2	1
3	SIN01	1	1	2	1	1	1	1	1	1	1	2	1	1	2
4	BRL01	1	1	2	1	1	1	2	1	1	4	2	1	1	2
5	BRL02	1	1	2	1	1	1	2	1	1	4	2	1	1	2
6	LCL02	1	2	2	1	1	1	2	1	1	5	2	1	1	1
7	LCL03	1	2	2	1	1	1	2	1	1	2	3	2	1	2
8	SIN02	1	2	2	1	1	1	2	1	1	3	2	2	1	2
9	RDL02	1	1	2	1	1	1	1	1	1	4	2	3	1	2
10	RTN01	1	1	2	1	1	1	2	1	1	3	2	2	1	2
11	LCL04	1	2	1	3	1	1	1	2	1	5	2	4	2	1
12	RDL03	1	1	2	1	1	1	1	1	1	1	2	3	1	2
13	SIN03	1	2	2	1	1	1	2	1	1	3	2	1	1	2
14	LCL05	1	1	2	1	2	1	1	1	1	2	2	1	1	1
15	MTP01	2	2	2	2	2	2	2	3	2	6	2	4	3	3
16	MTP02	2	2	2	2	2	2	2	3	2	6	2	4	3	3
17	MTP03	2	2	2	2	2	2	2	3	2	6	2	4	3	3
18	LCL06	1	2	1	1	1	1	1	1	1	1	2	1	1	1
19	LCL07	1	1	2	1	1	1	1	1	1	7	2	1	1	1
20	LCL08	1	1	2	1	1	1	1	1	1	3	3	3	1	1
21	LCL09	1	1	2	1	1	1	1	1	1	3	3	2	1	1

final extension of 5 min at 72 ºC. After completion of the cycling programme, reactions were held at 20 ºC. The amplified PCR products were electrophoresed by 3 % agarose gel containing 0.5 µg/mL ethidium bromide.

Data analysis

The alleles amplified by SSR and ISSR primers were scored for each primer across all genotypes. The number of alleles per locus, gene diversity, and polymorphism information content (PIC) were calculated using POPGENE version 1.31 (Yeh *et al*., 1999). Markers were scored as the presence (1) or absence (0) of bands, providing a data matrix used to calculate a genetic dissimilarity matrix (Jaccard's coefficient). The grouping among genotypes based on morphological traits and alleles was separately performed through the hierarchical unweighted pair-group method with arithmetic means analysis (UPGMA), and the statistical analysis was performed using statistical package for the social sciences (SPSS) version 16.0 (SPSS Inc., 1989 − 2007).

RESULTS

Morphological analysis

Phenotypic trait assessment of all examined *C. papaya* and mountain papaya accessions showed a variation in all qualitative traits assessed (Table 4). Out of all the genotypes there were 11 hermaphrodite plants and 10 female plants. All observed mountain papaya plants were hermaphrodite in the Shanthipura, Nuwara Eliya geographical area.

Mountain papaya plants showed a branching habit, while all *C. papaya* accessions exhibited single stems. The stem colour of the mountain papaya trees were greyish brown, while most of the *C. papaya* plants displayed light grey stems. The three main commercial *C. papaya* cultivars, Red Lady, Sinta and Rathne showed no consistent stem colour even along the same geographical region. The colour varied between greyish

brown to light grey. Pigmentation of the stem was indiscriminate for almost all the samples excluding a few local papaya genotypes (LCL04 and LCL06) and one Red Lady genotype (RDL01) found in the Kandy District. They showed a different pigmentation pattern, which is mostly confined to the uppermost part of the stem.

All *C. papaya* cultivars including commercially grown Sinta, Red Lady and Rathne showed similar leaf characters. They produced large palmate leaves with actinodromous venation, arranged in a spiral pattern and clustered in the upper section of the plant. Except two *C. papaya* genotypes, all the others possessed pale green leaf petioles. One local papaya genotype (LCL04) found in the Kurunegala District showed dark purple leaf petioles while another local genotype (LCL01) in the Kandy District had dark green leaf petioles, which was similar to the colour of mountain papaya leaf petioles. The blade of *C. papaya* leaves was divided into 9 main segments and had prominent yellowish mid ribs and veins, while mountain papaya and one local papaya accession (LCL05) from Dikkele, Kurunegala District showed leaves that were deeply divided into 7 main segments. Waxiness on the leaf surface was present only in mountain papaya plants.

The flower size, colour and the type of flowering showed similarities among *C. papaya* accessions. Their flowers were born on inflorescences, which appear in the axils of the leaves. All *C. papaya* cultivars bear cream colour flowers. Comparatively mountain papaya displayed distinct flower characters; they had small greenish colour flowers borne on inflorescences exhibited all along the trunk and showed dense inflorescences.

The fruit shape showed a wide dissimilarity among all papaya cultivars. Of all the cultivars, Red Lady and Sinta cultivars showed comparatively larger fruits, while mountain papaya had the smallest size fruits. Mountain papaya fruits showed five prominent broad, longitudinal ribs from the base to apex of the fruit (Figure 1). The

Table 4: SSR and ISSR primers used for PCR amplification

Primer	Motif	Sequence (5' → 3')	Size (bp)	T_A[a] (°C)
mCpCIR01	CT(18) GA(3)	F: ATCGTCTCCTTTTTCTGGTT		
		R: TCTGCCTCCCAATACACTAAT	100 – 300	54
mCpCIR02	TC(24)	F: AGCCACAACCTACGGGAAAT		
		R: AGTAACGGAGGAAAATGAGT	100 – 200	54
mCpCIR05	TC(18)	F: ATCGTCTCCTTTTTCTGGTT		
		R: TTCTGCCTCCCAATACACTA	100 – 300	54
mCpCIR08	CT(20) AC(5)	F: ACCCACCAGCAATCTCCAT		
		R: AGCAAACCACTCACTCTCATA	100 – 300	54
mCpCIR09	CT(9)	F: TGACGATAAAACCCTAACGA		
		R: TAAGAAACAGCGAAACCCTA	100 – 400	54
mCpCIR16	CT(9)	F: TACACTGCCTAACACCCATT		
		R: AACCAACCATAACTGCCTTT	200 – 400	54
mCpCIR17	GA(14)	F: ACAAACAAGTCCCCAAATCT		
		R: TACACTGCCTAACACCCATT	300 – 500	54
mCpCIR40	TC(13) TC(21)	F: TCGGTTCTCAGGTTTCTTCTAA		
		R: ACAATCACAGGCACACAT	100 – 300	52
mCpCIR45	GA(14)	F: AAAAGGACGAAAAGGAGACT		
		R: TTTGAACTACCTACACGAACT	200 – 400	52
S285	GAT(3)	F: AATGTGTGAGAATAGGTT		
		R: AATCTATCCTCCTCATGTA	200 – 400	50
S414	AC(7)	F: ATTCTTAGCCAGATGATGT		
		R: ATTGCATGTACACATACCGT	100 – 300	50
ISSR1	CA(GA)8	CA(GA)8	250 – 1200	57.4
ISSR2	GGGT(GGGGT)2G	GGGT(GGGGT)2G	300 – 1100	57.4

[a] Annealing temperature

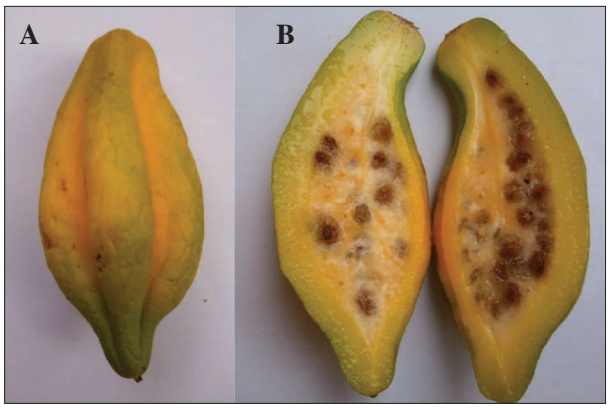

Figure 1: Ripen fruit of mountain papaya. A: External appearance of the fruit with prominent longitudinal ribs; B: longitudinal section of the fruit

peel colour of ripe *C. papaya* fruits ranged from light yellow to reddish orange, whereas mountain papaya fruits were light or pale yellow in colour. The flesh colour was distinct among all cultivars, but a clear separation based on it could not be seen in *C. papaya* cultivars. However, all three mountain papaya genotypes showed a distinct flesh colour, which was not similar to any of the *C. papaya* cultivars and they released a strong pleasant aroma when ripening, which was another highly distinct trait.

The UPGMA of morphological traits grouped the *C. papaya* and mountain papaya genotypes into two main clusters (Figure 2). One main cluster (cluster A) comprised all the mountain papaya genotypes collected from the Nuwara Eliya District (MTP 01−03) together with one Red Lady genotype (RDL 01) collected from

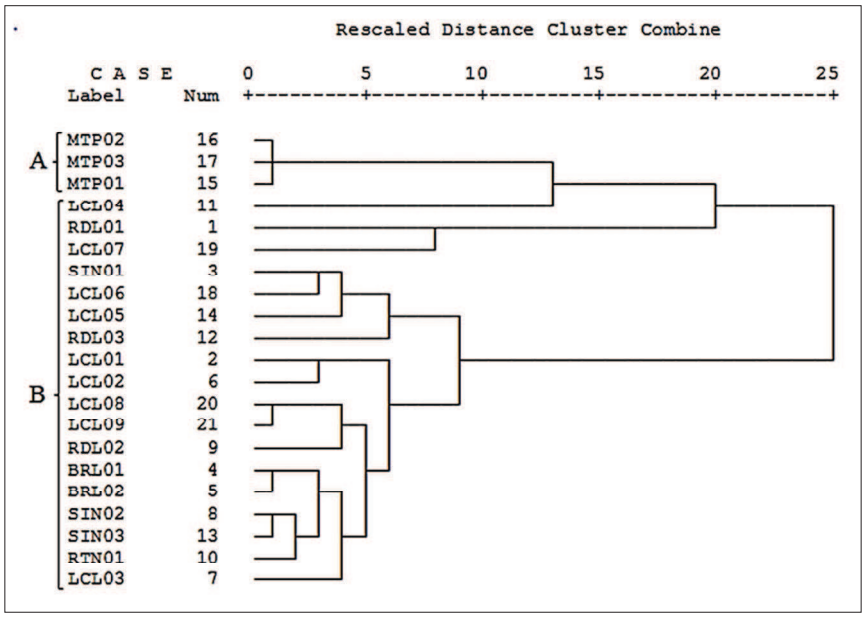

Figure 2: Dendrogram showing the relationships among *C. papaya* and mountain papaya germplasm, as obtained from morphological trait analysis using Eucledian distance with UPGMA algorithm.

the Kandy District and two local genotypes (LCL 04 and 07) collected from the Kurunegala District. All three mountain papaya accessions grouped into a single sub cluster in the main cluster A. LCL 04 shared similar characters with mountain papaya, while RDL 01 and LCL 07 shared more similar characters with each other. The other main cluster (cluster B) encompassed all the other genotypes that showed similarity. The morphological traits-based clustering pattern did not support any regional-wise separation.

SSR/ ISSR analysis

Thirteen primer pairs identified 33 alleles with an average frequency of 2.5 alleles per primer. Eight SSR primer pairs and two ISSR primer pairs (Figure 3) produced a total of 21 polymorphic alleles among the twenty one papaya genotypes assessed. SSR primers mCpCIR02, mCpCIR45 and S414 showed a monomorphic allele pattern, while SSR primers mCpCIR01, mCpCIR05, mCpCIR08, mCpCIR09, mCpCIR16, mCpCIR17,

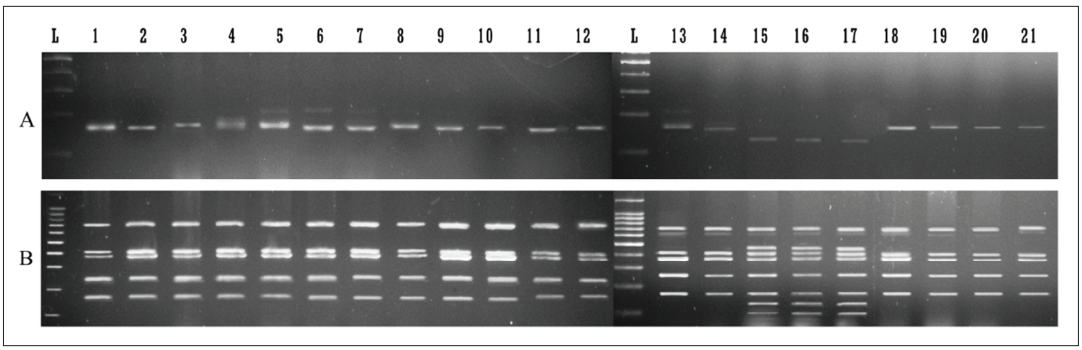

Figure 3: PCR amplification of 21 papaya samples with SSR and ISSR markers.
A: Primer mCpCIR05; B: primer ISSR01; lane L: 100 bp ladder; lane 1: RDL01; lane 2: LCL01; lane 3: SIN01; lane 4: BRL01; lane 5: BRL02; lane 6: LCL02; lane 7: LCL03; lane 8: SIN02; lane 9: RDL02; lane 10: RTN01; lane 11: LCL04; lane 12: RDL03; lane 13: SIN03; lane 14: LCL05; lane 15-17: MTP01-03; lane 18-21: LCL 06-09

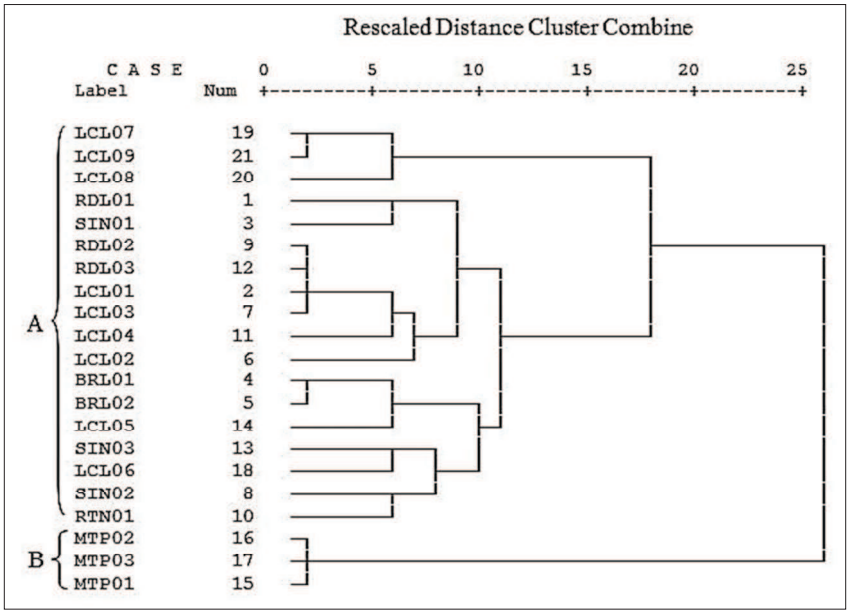

Figure 4: UPGMA dendrogram showing the relationships among *C. papaya* and mountain papaya germplasm, as obtained from SSR and ISSR marker analysis using Jaccard's coefficient.

mCpCIR40 and S285 showed polymorphism among mountain papaya and *C. papaya* accessions.

Twenty one accessions showed a mean gene diversity of 0.4611 with all the primers evaluated, and the primer mCpCIR08 showed the highest gene diversity of 0.93. SSR primers resulting in monomorphic allele pattern had the lowest gene diversity.

Major allele frequencies of each SSR and ISSR locus for the 21 accessions are presented in Table 5. Monomorphic primers had the highest major allele frequency, while the other primers had allele frequencies ranging from 0.1 to 0.9. The highest major allele frequency was observed with primers mCpCIR02, mCpCIR45 and S414 whereas the lowest allele frequency was with the primer ISSR 1.

The polymorphism information content (PIC) value provides an estimated figure of genetic diversity, and the average PIC of the 11 SSR and 2 ISSR loci in the 21 accessions was 0.3127. Marker mCpCIR08 had the highest PIC value of 0.5580 whereas markers mCpCIR40 and ISSR1 had the lowest PIC of 0.2356.

Table 5: Details of the allele polymorphism produced with SSR and ISSR markers

Primer	NSB [a]	NPB [b]	MAF [c]	GD [d]	PIC [e]
mCpCIR01	3	2	0.5682	0.2489	0.4908
mCpCIR02	1	0	1.0000	0.0000	0.0000
mCpCIR05	2	2	0.5000	0.6931	0.5000
mCpCIR08	3	3	0.5909	0.9369	0.5580
mCpCIR09	2	2	0.5909	0.6765	0.4836
mCpCIR16	2	2	0.6818	0.6255	0.4340
mCpCIR17	2	2	0.7273	0.5860	0.3967
mCpCIR40	2	2	0.8636	0.3983	0.2356
mCpCIR45	1	0	1.0000	0.0000	0.0000
S285	2	2	0.5455	0.6890	0.4959
S414	1	0	1.0000	0.0000	0.0000
ISSR1	8	3	0.1363	0.2489	0.2356
ISSR2	4	2	0.8636	0.8923	0.4616
Average	2.53	1.6923	0.6975	0.4611	0.3127

[a] Number of scored band; [b] number of polymorphic band; [c] major allele frequency; [d] gene diversity (Shannon's information index); [e] polymorphic information content

The UPGMA based on SSR and ISSR grouped the *C. papaya* and mountain papaya accessions into two main clusters (Figure 4). This result is described by the distinct polymorphic allele pattern between *C. papaya* and mountain papaya accessions obtained from mCpCIR01, mCpCIR40, ISSR1 and ISSR2 primers. Cluster A comprised all the *C. papaya* genotypes, while cluster B comprised 3 mountain papaya genotypes collected from the Nuwara Eliya District. Eighteen *C. papaya* genotypes in cluster A were further divided into several sub clusters. Local cultivars (LCL 07, 08 and 09) collected from three different locations in the Kurunagala District were sub-clustered separately in the main cluster A although such separation was not observed from morphological based clusters.

DISCUSSION

The genetic improvement of any crop is dependent on the utilisation of wild relatives, traditional varieties and modern breeding techniques. The assessment of genetic diversity at a given level is a requirement to select resistant and high yielding varieties (Mondini *et al*., 2009). In this study, the cluster analysis of morphological traits revealed that there is no distinct separation among the *C. papaya* genotypes grown in different geographic areas in Sri Lanka. Cultivar-wise separation could not be observed in most of the characters studied. However, it was revealed that mountain papaya is highly distinct from the *C. papaya* accessions although LCL 04 and LCL 07, and one Red Lady cultivar (RDL 01) shared similar morphological characters with mountain papaya accessions. Morphological markers alone are not reliable to analyse the intra-specific relationship between *C. papaya* and mountain papaya to examine the likelihood of intra-generic hybridisation. Therefore, DNA based molecular analysis was conducted as it is the preferred method for diversity analysis among plant breeders.

Molecular analysis showed clear information on inter- and intra-specific relationships giving estimates of the genetic relationship between *C. papaya* and mountain papaya, and among *C. papaya* cultivars. Thirty three alleles were identified by 11 SSR and 2 ISSR markers proving their ability to be used as polymorphic markers in both *C. papaya* and mountain papaya accessions. Although RAPD markers have been extensively used to assess some traits in papaya (Sondur *et al*., 1996; Jobin-Decor *et al*., 1997), there are only a few reports in papaya analysed by SSR and ISSR (Carrasco *et al*., 2009; de Oliveira *et al*., 2010), which are more reliable and deliver re-producible results. In this study, mCpCIR08 showed the highest PIC value thereby indicating its usefulness in the screening of *C. papaya* accessions. DNA based cluster analysis also revealed that *C. papaya* cultivars are not much divergent from each other, which may be due to sharing the same alleles in most of the SSR and ISSR markers. Results of the molecular analysis revealed that mountain papaya and *C. papaya* are genetically distant relatives. However, out of the 33 alleles produced, 12 alleles were common for both *C. papaya* and mountain papaya and hence, it can be assumed that mountain papaya could be more closely aligned to *C. papaya*. Drew *et al*. (1998) have reported the possibility of inter-generic hybridisation between some species of the genus, *Vasconcelleae* and *C. papaya,* proving their close relationship.

Vasconcellea cundinamarcensis, V. cauliflora, V. quercifolia and *V. stipulata* are considered as the major sources for extreme resistance to PRSV-P (Manshardt & Wenslaff, 1989; Drew *et al.*, 1998). Resistance to PRSV-P in *C. papaya* has not been identified to date. Inter-generic hybridisation conducted between *Vasconcellea* species and *C. papaya* has yielded resistant hybrids indicating the possibility of donating PRSV-P resistant genes (Manshardt & Wenslaff, 1989; Drew *et al*., 1998). However, these hybrids were not commercially valuable due to the presence of unfavourable traits. Therefore, it is necessary to continue backcross breeding with *C. papaya* as the recurrent parent to maximise the *C. papaya* genetic background while minimising the region of donor DNA to the target loci of PRSV-P resistance inherited from

Vasconcellea species. This backcross breeding is only possible with genetic markers that are present throughout the genome. Therefore, finding out polymorphic markers between mountain papaya and *C. papaya* is important to produce cultivars with PRSV-P virus resistance, cold resistance etc., *via* marker assisted backcrossing utilising mountain papaya genetic resource.

CONCLUSION

Morphological and molecular marker-based cluster analysis revealed that there was no clear genetic separation among the *C. papaya* cultivars grown in different geographic areas in Sri Lanka. SSR markers: mCpCIR02, mCpCIR45 and S414 showed a monomorphic allele pattern in 3 % agarose, while SSR markers: mCpCIR01, mCpCIR05, mCpCIR08, mCpCIR09, mCpCIR16, mCpCIR17, mCpCIR40, and S285 and ISSR markers: ISSR01 and ISSR02 were polymorphic between mountain papaya and *C. papaya* accessions. Out of the 13 markers tested, SSR marker mCpCIR08 would be useful in screening *C. papaya* accessions due to the highest PIC value. Both morphological and molecular analysis proved that mountain papaya and *C. papaya* are genetically distant relatives. However, the presence of common genetic and morphological features between *C. papaya* and mountain papaya suggest the possibility of inter-specific hybridisation.

Acknowledgement

The authors express their gratitude to the Wayamba University of Sri Lanka (Research Grant No. : SRHDC/RP/04/13-17) for giving financial support for this research.

REFERENCES

1. Badillo V.M. (2000). *Carica* L. *vs*. *Vasconcella* St.-Hil. (Caricaceae) con la rehabilitacionde este ultimo. *Ernstia* **10**: 74 – 79.
2. Carrasco B., Avila P., Perez-Diaz J., Mun˜oz P., Garcı´a R., Lavandero B., Zurita-Silva A., Retamales J.B. & Caligari P.D.S. (2009). Genetic structure of highland papayas (*Vasconcellea pubescens*) cultivated along a geographic gradient in Chile as revealed by inter simple sequence repeats (ISSR). *Genetic Resources and Crop Evolution* **56**: 331 – 337.
DOI: https://doi.org/10.1007/s10722-008-9367-1
3. Carvalho F.A., Filer D. & Renner S.S. (2015). Taxonomy in the electronic age and an e-monograph of the papaya family (Caricaceae) as an example. *Cladistics* **31**: 321 – 329.
4. da Costa F.R., Pereira T.N., Gabriel A.P.C. & Pereira M.G. (2011). ISSR markers for genetic relationships in Caricaceae and sex differentiation in papaya. *Crop Breeding and Applied Biotechnology* **11**: 352 – 357.
DOI: https://doi.org/10.1590/S1984-70332011000400009
5. Dassanayake M.D., Fosberg F.R. & Clayton W.D. (1995). *Revised Handbook to the Flora of Ceylon*, volumes VIII-IX. Amerind Publishing Co. Pvt. Ltd., New Delhi, India.
6. de Oliveira E.J., Silva A.D.S., de Carvalho F.M., dos Santos L.F., Costa J.L., Amorium V.B.D.O. & Dantas J.L.L. (2010). Polymorphic microsatellite marker set for *Carica papaya* L. and its use in molecular-assisted selection. *Euphytica* **173**: 279 – 287.
DOI: https://doi.org/10.1007/s10681-010-0150-y
7. Dillon S., Ramage C., Ashmore S. & Drew R.A. (2006). Development of a codominant CAPS marker linked to PRSV-P resistance in highland papaya. *Theoretical and Applied Genetics* **113**(6): 1159 – 1169.
DOI: https://doi.org/10.1007/s00122-006-0375-2
8. Department of Agriculture (DOA) (2014). *Agstat. Pocket Book of Agricultural Statistics*. Department of Agriculture, Peradeniya.
9. Doyle J.J. & Doyle J.L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemistry Bulletin* **19**: 11 – 15.
10. Drew R.A., O'Brien C.M. & Magdalita P.M. (1998). Development of interspecific *Carica* hybrids. *Acta Horticulturae* **461**: 285 – 292.
DOI: https://doi.org/10.17660/ActaHortic.1998.461.31
11. International Board for Plant Genetic Resources (IBPGR) (1988). *Descriptors for Papaya*. International Board for Plant Genetic Resources, Rome, Italy.
12. Jiménez V.M., Mora E. & Gutiérrez V.M. (2012). *Biology of the Papaya Plant*. Available at *http://www.cultivopapaya.org/wp-content/uploads/9781461480860-c1.pdf,* Accessed 15 May 2014.
13. Jobin-Décor M.P., Graham G.C., Henry R.J. & Drew R.A. (1997). RAPD and isozyme analysis of genetic relationships between *Carica papaya* and wild relatives. *Genetic Resources and Crop Evolution* **44**: 471 – 477.
14. Madarbokus S. & Ranghoo-Sanmukhiya V.M. (2012). Identification of genetic diversity among papaya varieties in Mauritius using morphological and molecular markers. *International Journal of Life Sciences Biotechnology and Pharma Research* **1**(4): 153 – 163.
15. Manshardt R.M. & Wenslaff T. (1989). Interspecific hybridization of papaya with other *Carica* species. *Journal of the American Society for Horticultural Science* **114**: 689 – 694.
16. Mondini L., Noorani A. & Pagnotta M.A. (2009). Assessing plant genetic diversity by molecular tools. *Diversity* **1**: 19 – 35.
DOI: https://doi.org/10.3390/d1010019
17. Moussaoui A., Nijs M., Paul C., Wintjens R., Vincentelli J., Azarkan M. & Looze Y. (2001). Revisiting the enzymes stored in the laticifers of *Carica papaya* in the context of their possible participation in the plant defense mechanism. *Cell and Molecular Life Sciences* **58**(4): 556 – 570.
DOI: https://doi.org/10.1007/PL00000881

18. Muthulakshmi S., Balamohan T.N., Amutha R., Baby Rani W., Mareeswari P. & Indira K. (2007). Breeding for cold tolerance in papaya (*Carica papaya* L.) through hybridization. *Research Journal of Agriculture and Biological Sciences* **3**(4): 264 – 266.
19. O'Brien C.M. & Drew R.A. (2010). Marker-assisted hybridization and backcrossing between *Vasconcellea* species and *Carica papaya* for PRSV-P resistance. *Acta Horticulturae* **859**: 361 – 368.
DOI: https://doi.org/10.17660/ActaHortic.2010.859.43
20. Ramos H.C.C., Pereira M.G., Silva F.F., Goncalves L.S.A., Pinto F.O., de Souza Filho G.A. & Pereira T.S.N. (2011). Genetic characterization of papaya plants (*Carica papaya* L.) derived from the first backcross generation. *Genetics and Molecular Research* **10**(1): 393 – 403.
DOI: https://doi.org/10.4238/vol10-1gmr1004
21. Senaratna L.K. (2001). *A Check List of the Flowering Plants of Sri Lanka*, 1st edition, pp. 77. 47/5, National Science Foundation, Maitland Place, Colombo 07, Sri Lanka.
22. Sondur S.N., Manshardt R.M. & Stiles J.I. (1996). A genetic linkage map of papaya based on randomly amplified polymorphic DNA markers. *Theoretical and Applied Genetics* **93**: 547 – 553.
DOI: https://doi.org/10.1007/BF00417946
23. Storey W.A. (1976). *Evolution of Crop Plants*. Longman, London, UK.
24. Weerakoon D., Goonatilake S.A., Weeratunge V., Perera N. & Chandranimal D. (2009). *Halgolla Estate, Kelani Valley Plantation Management Plan for the Local Nature Reserve*. International Union for Conservation of Nature, Sri Lanka.
25. Yeh F.C., Yang R. & Boyle T. (1999). User Manual for POPGENE, version 1.31, *Microsoft Window-Based Freeware for Population Genetic Analysis*. A joint Project Development by Francis C. Yeh and Rong-Cai Yang, University of Alberta, and Tim Boyle, Centre for International Forestry Research, Indonesia.