Agr. Nat. Resour. 53 (2019) 218–227

AGRICULTURE AND NATURAL RESOURCES

Journal homepage: http://anres.kasetsart.org

Research article

Diversity of durian (*Durio zibethinus* L.) from Nonthaburi, Thailand based on morpho-palatability characteristics and simple sequence repeat markers

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Article Info

Article history:
Received 16 March 2018
Revised 9 July 2018
Accepted 11 July 2018
Available online 30 June 2019

Keywords:
Intraspecific variation,
Morphometrics,
Microsatellite markers,
Phenogram,
Simple sequence repeats (SSRs)

Abstract

Durian is a high-value, tropical fruit well known for its unique, pungent smell and sweet and creamy flesh. Nonthaburi province in central Thailand has been known as a source of top-quality durian cultivars, especially Mon Thong. However, with the recent urbanization and frequent severe weather conditions, these valuable local cultivars are disappearing at an alarming rate. Therefore, there is an urgent need for morpho-palatability as well as molecular information in order to correctly identify and characterize each cultivar. This study was the first to report durian diversity in Thailand using both morpho-palatability characteristics and molecular marker analysis. Morpho-palatability diversity was assessed in 31 cultivars using a modified descriptor for durian that measured 22 qualitative traits and 33 quantitative traits from the leaves, flowers and fruits. In addition, 24 cultivars were used for genetic diversity analysis based on simple sequence repeat (SSR) markers. The diversity of durian cultivars could be seen from the morphometric analysis. As expected, several cultivars showed unique characters that could be used for future breeding purposes. It was also found that certain characters were more informative than others at characterizing durian cultivars, which could lead to an improvement of cultivar identification. SSR marker profiles were considerably informative in distinguishing cultivars. Molecular data analysis showed that most cultivars were clustered together, with only a few in small, separate clusters. The results from this study should help to improve durian cultivar conservation and breeding for better quality.

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https://doi.org/10.34044/j.anres.2019.53.3.02

Introduction

Durian (*Durio zibethinus* L.), a large fruit with strong, rich, sulfury smell and sweet creamy flesh, is highly popular in Southeast Asia and gaining popularity elsewhere around the world (Brown, 1997). The highly nutritional flesh and the big, sharp spines resembling the spiny crown of kings had earned durian its nickname as the "*King of tropical fruit*" (Subhadrabandhu and Ketsa, 2001).

The *Durio* genus, in the family *Malvaceae,* consists of 27 species, of which six produce edible fruits (Watson, 1984). Durian originated and has been grown in the warm, wet conditions of the equatorial tropics and is cultivated in Southeast Asia, particularly in Thailand, Malaysia, Indonesia and the Philippines (Nanthachai, 1994). The existing commercial cultivars of durian arose from natural selection and artificial breeding by growers and later by breeders (Songnuan et al., 2014).

Over 200 durian cultivars have been named and recognized in Thailand (Somsri, 2007). The majority of the most famous commercial cultivars, including Mon Thong, originated in Nonthaburi province, where, owing to the fertile soil of the Chao Phraya River Basin, this province has been renowned for its durian orchards since the 14th century (De La Loubère, 1986). With the large number of cultivars, each with its unique quality that can command a high price (as much as 250 USD per fruit according to Songnuan et al., 2014), there is a pressing need for correct identification and characterization of each durian cultivar.

Folk grouping is primarily based on morphological characteristics that can only be discerned by trained experts and is often indescribable and arguable. Previous studies had grouped durian cultivars into six groups—Kob, Luang, Kan Yao, Kampan, Thong Yoi and Miscellaneous—based on morphological characteristics (Hiranpradit et al., 1992; Kittiwarodom and Jutamanee, 2011). In addition, genetic availability among cultivars of durian from Nonthaburi province had been studied using random amplified polymorphic DNA (RAPD) markers (Vanijajiva, 2011), inter-simple sequence repeats (ISSR) markers (Vanijajiva, 2012) and amplified fragment length polymorphism (AFLP) (Kittiwarodom and Jutamanee, 2011).

Microsatellites are highly polymorphic, short tandem repeats of nucleotides which are assumed to be randomly distributed throughout the nDNA, cpDNA and mtDNA. Their hypervariability and ubiquitous occurrence make them useful markers for high resolution of genetic diversity, evolution and the phylogenetic relationship within and between species and populations (Ellegren, 2004; Lowe et al., 2004; Feng et al., 2006).

The objective of the current study was to determine the genetic variation, diversity and relatedness of durian cultivars from Nonthaburi province using both morpho-palatability characteristics and SSR markers. This information will provide an understanding that can lead to the identification, classification and conservation of rare durian cultivars.

Materials and Methods

Plant material and sampling sites

In total, 42 durian cultivars were sampled from Mueang, Bang Kruai, Bang Yai and Pak Kret districts, Nonthaburi province, Thailand, in 2010 (Table 1). Leaves, flowers and fruits of 31 cultivars were collected and photographed to investigate their morpho-palatability characteristics. Due to limited availability, not all types of samples were available for all cultivars mainly due to the 2011 Great Flooding which destroyed most of the Nonthaburi durian orchards (Songnuan et al., 2014). In fact, some cultivars, such as Ma Fo, were devastated and are now believed to be extinct (a gardener, Suang Ketkrai, pers. comm.). SSR analysis was performed on 24 cultivars, 14 of which had available morpho-palatability information.

Morpho-palatability characteristics

To study the variation of the morpho-palatability characteristics of durian, data were collected based on a descriptor modified from Descriptors for Durian (Bioversity, 2007). In total, 22 qualitative traits (Table 2) and 33 quantitative traits (Table 3) were determined.

The qualitative traits of samples were categorized and examined for their morphological diversity. The data were transformed into a numerical matrix and based on Shannon's information index was used to calculate the variation among cultivars in each trait using GENAIEx 6.5 (Peakall and Smouse, 2012). The discriminating power (D_j) which indicates the ability of each trait to index true individual differences was calculated as $D_j = 1–C_j$, where C_j is the confusion probability (the probability that two randomly selected cultivars have the identical states for a given trait). C_j was was defined using Equation 1 according to Tessier et al. (1999):

$$C_j = \sum_{k=1}^{K} P_k \frac{Npk-1}{N-1} \qquad (1)$$

P_k where P_k is the frequency of the k^{th} state, N is the sample size and K is the total number of states of the j^{th} trait.

Thirty-three quantitative traits of durian from the leaves, flowers, fruits and seeds were measured. The quantitative data were also transformed into a numerical matrix and Pearson's correlation coefficient was calculated among all traits using the PASW statistic 18 software (SPSS Inc., 2009).

DNA isolation

Genomic DNA was isolated from young leaves using the Dneasy® Plant Mini Kit (Qiagen; Germantown, MD, USA). DNA quality was examined using 1% agarose-gel electrophoresis, and the DNA concentration was estimated using a spectrophotometer DQ 200 (Hoefer, Holliston, MA, USA). DNA samples were diluted to a concentration of 10 ng/µL using ddH_2O and stored at -20°C.

Table 1 Forty-two durian cultivars collected from Nonthaburi province, Thailand used in this study.

No.	Acc. No.	Durian cultivar	Location	Cultivar group
1	01	Kop Wat Kluai	Bang Si Thong, Bang Kruai	Kop
2	04	Kathoei Nuea Lueang	Bang Krang, Mueang	Miscellaneous
3	05	Chomphu Si	Bang Si Thong, Bang Kruai	Luang
4	07	Chat Si Thong	Bang Si Thong, Bang Kruai	Thong Yoi
5	08	Kop Ta Thao	Bang Si Thong, Bang Kruai	Kop
6	09	Kop Ta Kham	Bang Si Thong, Bang Kruai	Kop
7	10	E-Luang	Bang Si Thong, Bang Kruai	Laung
8	11	Kop Mae Thao	Bang Si Thong, Bang Kruai	Kop
9	12	Kop Sao Noi	Bang Si Thong, Bang Kruai	Kop
10	13	Chani	Bang Si Thong, Bang Kruai	Kop
11	14	Thong Yoi Chat	Bang Si Thong, Bang Kruai	Thong yoi
12	15	Kan Yao	Bang Si Thong, Bang Kruai	Kan Yao
13	16	Met Nai Yai Prang	Bang Krang, Mueang	Miscellaneous
14	17	Mon Thong	Bang Krang, Mueang	Kampan
15	18	Kampan Doem	Bang Rak Noi, Mueang	Kampan
16	19	Yammawat	Bang Rak Noi, Mueang	Laung
17	21	Sao Chom	Bang Rak Noi, Mueang	Miscellaneous
18	24	Chao Ngo	Bang Rak Noi, Mueang	Miscellaneous
19	27	Kathoei Nuea Khao	Bang Krang, Mueang	Miscellaneous
20	28	Keng Thong	Bang Krang, Mueang	Miscellaneous
21	30	Kop Chai Nam	Bang Len, Bang Yai	Kop
22	34	Daeng Ratsami	Bang Len, Bang Yai	Thong Yoi
23	35	Kradum Khieo	Pak Kret, Pak Kret	Miscellaneous
24	36	Sao Noi Ruen Ngam	Pak Kret, Pak Kret	Miscellaneous
25	37	Ma Fo	Pak Kret, Pak Kret	Miscellaneous
26	38	Kampan Nuea Lueang	Pak Kret, Pak Kret	Kampan
27	39	Bat Thong Kham	Khlong Phra Udom, Pak Kret	Miscellaneous
28	41	Kop Ta Thuam	Khlong Phra Udom, Pak Kret	Kop
29	43	Kampan Phuang	Khlong Phra Udom, Pak Kret	Kampan
30	44	Kampan Chao Krom	Khlong Phra Udom, Pak Kret	Kampan
31	45	Chai Ma Fai	Bang Krang, Mueang	Kampan
32	48	Kop Wai	Bang Krang, Mueang	Kop
33	51	Kop Si Nak	Bang Si Thong, Bang Kruai	Kop
34	52	Sao Chom Khieo	Wat Chalo, Bang Kruai	Miscellaneous
35	54	Daeng Ratsami	Wat Chalo, Bang Kruai	Thong Yoi
36	55	Kop Kan Lueang	Wat Chalo, Bang Kruai	Kop
37	60	Kop Phikun	Bang Si Thong, Bang Kruai	Kop
38	62	Kop Champa	Nonthaburi local market	Kop
39	63	Kop Ta Klom	Nonthaburi local market	Kop
40	64	Kop Ta Khao	Nonthaburi local market	Kop
41	65	Kop Si Nuan	Nonthaburi local market	Kop
42	66	Kan Yao Song Huat	Nonthaburi local market	Kan Yao

Table 2 Categorical trait, proportion of occurrence, number of observed states (*Kj*), Shannon's information index (*Hj*) and discriminating power (*Dj*) of 22 qualitative morphological-palatability trait in 31 durian cultivars from Nonthaburi province, Thailand

No.	Code	Traits	Category					*Kj*	*Hj*	*Dj*
1	LUC	Leaf upper surface color	(1)146A [.56]	(2)147 [.44]				2	0.686	0.513
2	LLC	Leaf lower surface color	(1)148B [.20]	(2)152A [.80]				2	0.500	0.333
3	LAS	Leaf apex shape	(1)acute [.00]	(2)acuminate [1.0]	(3)long acuminate [.00]	(4)caudate [.00]	(5)cuspidate [.00]	1	-	-
4	LBS	Leaf base shape	(1)round [.28]	(2)obtuse [.00]	(3)acute [.72]	(4)cuneate [.00]	(5)cordate [.00]	2	0.593	0.420
5	CTA	Calyx tooth apex	(1)acuminate [.24]	(2)acute [.48]	(3)round [.29]			3	1.053	0.667
6	STYS	Style shape	(1)straight [.00]	(2)curved [1.0]	(3)wavy [.00]	(4)straight and hooked [.00]		1	-	-
7	STGS	Stigma shape	(1)turbinate [.00]	(2)capitate, not lobed [.00]	(3)capitate, 5 lobed [1.0]			1	-	-
8	FAS	Fruit apex shape	(1)pointed [.39]	(2)convex [.32]	(3)mammiform [.13]	(4)truncate [.16]	(5)depressed [.00]	4	1.291	0.727
9	FBS	Fruit base shape	(1)depressed [.10] (6)acute [.10]	(2)necked [.03] (7)other [.00]	(3)convex [.45]	(4)truncate [.29]	(5)concave [.00]	6	1.392	0.714
10	FSS	Fruit spine shape	(1)hooked [.10] (6)conical [.06]	(2)convex [.16] (7)pyramidal [.13]	(3)pointed-convex [.10] (8)other [.03]	(4)concave [.16]	(5)pointed-concave [.26]	8	1.942	0.869
11	FSTC	Fruit stalk color	(1)165B [.03]	(2)165C [.03]	(3)199A [.71]	(4)199B [.13]	(5)200C [.10]	5	0.955	0.484
12	FSKC	Fruit skin color	(1)144A [.10] (6)148B [.10] (11)161A [.03]	(2)146A [.03] (7)152A [.10]	(3)146B [.13] (8)152B [.10]	(4)146C [.16] (9)152C [.10]	(5)148A [.06] (10)152D [.10]	11	2.313	0.925
13	ATE	Aril texture	(1)soft [.33]	(2)intermediate [.37]	(3)firm [.30]			3	1.094	0.689
14	AJ	Aril juiciness	(1)juicy [.37]	(2)non-juicy [.63]				2	0.659	0.484
15	FIB	Presence of fiber	(1)low [.26]	(2)medium [.52]	(3)high [.22]			3	1.025	0.638
16	FCR	Flesh creaminess	(1)poor [.25]	(2)fair [.36]	(3)medium [.25]	(4)strong [.14]		4	1.339	0.754
17	FT	Flesh taste	(1)slightly sweet [.18]	(2)sweet [.61]	(3)sweet with bitter after taste [.14]	(4)bitter sweet [.07]		4	1.077	0.595
18	FA	Flesh aroma	(1)aroma [.25]	(2)moderate [.64]	(3)strong [.11]			3	0.870	0.532
19	FC	Flesh color	(1)5D [.03] (6)10D [.03] (11)12C [.03] (16)15D [.10]	(2)6D [.03] (7)11A [.07] (12)13B [.10] (17)16C [.03]	(3)8C [.03] (8)11B [.14] (13)13C [.03] (18)20A [.03]	(4)9D [.03] (9)11D [.03] (14)14C [.10] (19)21D [.03]	(5)10C [.03] (10)12A [.03] (15)14D [.03]	19	2.787	0.961
20	SPL	Easiness of splitting	(1)easy [.11]	(2)moderate [.68]	(3)hard [.21]			3	0.833	0.500
21	FS	Flesh stickiness	(1)low [.82]	(2)moderate [.14]	(3)high [.04]			3	0.559	0.315
22	SC	Seed coat color	(1)19B [.03] (6)165D [.03]	(2)164A [.07]	(3)164B [.38]	(4)165B [.41]	(5)165C [.07]	6	1.334	0.697

Kj = number of observed states; *Hj* = Shannon's information index; *Dj* = discriminating power

Table 3 Mean, standard deviation (SD), range and coefficient of variation (%CV) of 33 quantitative traits in 31 durian cultivars from Nonthaburi province, Thailand

No.	Part	Code	Trait	Mean	SD	Range	%CV
1	Leaf	LBL	Leaf blade length (cm)	14.18	2.58	6.70–19.20	18.20
2		LBW	Leaf blade width (cm)	4.80	1.06	3.10–7.60	22.03
3		LBSR	Leaf blade shape (length/width)	3.03	0.63	1.58–3.92	20.72
4		PETL	Petiole length (cm)	1.85	0.35	0.80–2.70	18.97
5	Flower	FBL	Flower bud length (cm)	2.43	0.51	1.50–3.80	21.09
6		FBW	Flower bud width (cm)	1.76	0.21	1.30–2.00	11.70
7		FBSR	Flower bud shape (length/width)	1.38	0.22	1.00–2.11	15.98
8		BL	Bract length (cm)	2.53	0.24	2.10–2.90	9.53
9		BW	Bract width (cm)	1.93	0.31	1.40–2.60	16.13
10		CL	Calyx length (cm)	2.62	0.39	1.90–3.50	14.83
11		CW	Calyx width (cm)	2.16	0.32	1.60–2.70	14.71
12		CS	Calyx shape (length/width)	1.24	0.17	1.00–1.53	13.87
13		PEDL	Pedicel length (cm)	4.54	0.81	3.30–5.80	17.85
14		PW	Petal width (cm)	2.32	0.57	1.50–3.40	24.49
15		PS	Petal shape (length/width)	2.04	0.48	1.21–2.87	23.55
16		STL	Style length (cm)	4.97	0.56	4.10–5.90	11.31
17	Fruit	FWE	Fruit weight (kg)	2.36	1.03	1.06–5.33	43.64
18		FL	Fruit length (cm)	21.55	4.40	14.70–35.30	20.44
19		FSLW	Fruit shape (length/width)	1.17	0.18	0.86–1.48	15.25
20		FSDD	Fruit shape (diameter/depth)	1.13	0.11	1.00–1.45	9.83
21		FSTL	Fruit stalk length (cm)	6.05	1.28	4.04–10.52	21.09
22		FSPD	Fruit spine density (no. of spines/25 cm^2)	14.76	5.92	5.67–30.33	40.09
23		FSPL	Fruit spine length (cm)	1.48	0.28	0.98–2.10	19.23
24		FRT	Fruit rind thickness (cm)	1.87	0.58	0.83–2.84	30.96
25		AT	Aril thickness (cm)	1.28	0.51	0.35–2.60	39.52
26		CARP	Number of carpels/fruit	5.19	0.48	4.00–6.00	9.19
27		EFC	Edible flesh content (%)	32.13	8.14	15.39–47.17	25.35
28	Seed	SWE	Seed weight (g)	25.89	6.28	16.96–38.99	24.27
29		SL	Seed length (cm)	5.81	1.09	4.47–9.30	18.82
30		SW	Seed width (cm)	3.45	0.60	2.47–5.97	17.51
31		SS	Seed shape (length/width)	1.61	0.37	0.64–2.46	23.07
32		MS	Number of mature seeds/fruit	6.83	5.11	0.00–20.00	74.89
33		IMMS	Number of immature seeds/fruit	3.07	2.74	0.00–13.00	89.21

Polymerase chain reaction amplification, electrophoresis and silver staining

The nine SSR markers (Table 4) used for polymerase chain reaction (PCR) amplification in this study were selected from 20 markers investigated by Songnuan et al. (2014). PCR was carried out in a 10 µL volume containing 2 µL of 10 ng/µL of genomic DNA template, 1 µL of 10X PCR buffer (10X Taq PCR buffer with Mg^{2+}), 0.3 µL of 50 mM $MgCl_2$, 0.1 µL of Taq DNA polymerase (Fermentas, Waltham, MA, USA), 0.3 µL of 10 µM of forward and reverse primers, 4 µL of ddH_2O and 2 µL of 1 mM dNTPs. Depending on the primer pair use, DNA amplification was performed in a PCR thermal cycle 9600 (Perkin-Elmer, Waltham, MA, USA), programmed for an initial melting step at 95°C for 3 min, followed by 10 cycles, each cycle consisting of three steps at 94°C for 1 min, 65°C for 1 min, reducing by 0.5°C /cycle for the next nine cycles, 72 °C for 90 sec, followed by 30 cycles, each cycle consisting of three steps of 94°C 1 min, 55°C for 1 min and 72°C for 90 sec. A final extension step was done at 72°C for 7 min.

After the steps of amplification, samples were mixed with a half volume of gel loading buffer (98% formamide, 10 mM ethylenediaminetetraacetic acid, pH 8, 0.25% xylene cyanol as tracking dye), heated for 5 min at 94°C, chilled quickly on ice and run on 4.5% denaturing polyacrylamide gels (acrylamide and bis-acrylamide; Seqni-Gen® GT Nucleic Acid Electrophoresis Cell) from BIO-RAD (Hercules, CA, USA) using the method described by Bassam et al. (1991). Gel electrophoresis was performed at 50 W of constant power for 90 min, in 1X TBE buffer as the running buffer. The gels were visualized using silver staining.

Table 4 Primer sequence, size range, number of alleles per locus (N_A), observed heterozygosity (H_o), expected heterozygosity (H_e) and polymorphism information content (*PIC*) of nine microsatellite loci used in this study

Primer	Primer sequence (5'-3')	Size range (bp)	N_A	H_o	H_e	*PIC*
MS1CT-5	F: CCT GCA AAA CCA AAC CAA AT	245-275	2	0.500	0.479	0.329
	R: CAA AGG GAG TAT CCT TCC AG					
MS1CT-6	F: TAA ACT GGC AAT GAA ACA GC	146-158	3	0.682	0.498	0.417
	R: CCA AAC AGC TAA ACC CAT GA					
MS1CT-12	F: GAC GAC ACC AGC GAT CAA C	195-205	3	0.391	0.629	0.535
	R: ATG GCG TCA TTT TGC TTT TC					
MS1GT-15	F: CCA AAC AGC TAA ACC CAT G	185-197	3	0.708	0.513	0.435
	R: TGC AAG AGA AGT TGT GTA TCT GG					
MS1GT-19	F: TGA GTG GCG CAC TAA AAC AC	230-236	2	0.292	0.311	0.258
	R: AGG TGT CTC AGC TGG TTT GC					
MS1GT-22	F: ACC ATC AAC GGT CAA AGG TT	175-180	2	0.636	0.495	0.367
	R: TGT ACA GAA GCC AAA AGA AAA AC					
MS1GT-27	F: CAA TGC TTC CAG GTT TCC AT	203-205	2	0.588	0.428	0.329
	R: CCT GGC AGG GGG TTA TTT AT					
MS1AAC-5	F: AAT CCT TCA ACC CAC ACC AA	207-235	2	0.600	0.431	0.332
	R: TTC TTT TCG CCA GAA ACA GC					
MS1AAC-19	F: AGC CCA TTT GGT GCT GTA AT	220-226	2	0.667	0.496	0.368
	R: AGC AAC CTC AGC CAT TGT TT					

Molecular data analysis

The presence or absence of each band was scored as 1 or 0, respectively. The number of alleles per locus was determined. The observed heterozygosity and the expected heterozygosity were calculated using GenAlEx 6.5 to examine the genetic variation among cultivars. The polymorphism information content (PIC) was also used to assess the genetic diversity using CERVUS 3.0 (Kalinowski et al., 2007), where PIC > 0.5 was considered as highly informative, 0.5 > PIC > 0.25 was reasonably informative and PIC < 0.25 was slightly informative (Anderson et al., 1993).

Cluster analysis

The dendrograms based on the morpho-palatability characteristics and molecular data were constructed using UPGMA (unweighted pair-group method with arithmetic mean) clustering and the cophenetic correlation coefficient (r) of the dendrogram was calculated using the software, NTSYS-pc version 2.1 (Rohlf, 2000). Based on the band analysis, a method yielding a high r value can be considered as an appropriate method for a particular analysis (Romesburg, 1984). The degree of fit can be interpreted subjectively as, $0.9 \leq r$, as a very good fit, $0.8 \leq r < 0.9$ as a good fit, $0.7 \leq r < 0.8$ as a poor fit and $r < 0.7$ as a very poor fit (Rohlf, 1992).

Results

Morpho-Palatability characteristics

A considerable level of diversity was identified in the durian cultivars from Nonthaburi, Thailand at both the morpho-palatability and molecular levels. In total, 31 cultivars were sampled and assessed for 22 qualitative and 33 quantitative traits. They exhibited different numbers of observed states that varied from 1 to 11. The leaf apex, style and stigma shape were non-informative traits while characters involving shapes such as fruit spine shape and fruit base shape were highly polymorphic. The number of observed states for each trait and the proportion of each state are shown in Table 2.

All polymorphic traits were used to analyze the variation among cultivars in each trait based on Shannon's information index (H_j) as well as the discriminating power (D_j). The H_j value was high for flesh color followed by fruit skin color and fruit spine shape (2.787, 2.313 and 1.942, respectively).

The 33 quantitative traits investigated were: 4 leaf traits, 12 flower traits, 11 fruit traits and 6 seed traits. The overall standard deviation, which indicated the amount of variation in each trait among cultivars, was relatively low compared to the mean of the dataset. However, high variation was found for the number of mature and immature seeds per fruit.

The variability of the quantitative traits was also determined using the coefficient of variation (%CV) which is shown in Table 3. Fruit weight, fruit spine density, aril thickness, seed width and fruit rind thickness had relatively high variation among the durian cultivars, while the number of carpels per fruit, bract length and fruit shape (diameter/depth) had low variability.

Nine from 33 quantitative traits were selected for representation using histograms in Fig. 1 because they are commonly used by local gardeners to identify specific cultivars or are of interest to consumers or both (Hiranpradit et al., 1992). These nine features were: leaf blade shape (leaf-to-width ratio [L/W]), petiole length, fruit weight, fruit stalk length, fruit spine density, fruit spine length, aril thickness, fruit rind thickness and edible flesh content. Some durian cultivars had outstanding values in these traits, for example Chomphu Si had the lowest leaf blade length and L/W ratio (Fig. 1A, arrows). In addition, the petiole length of Chomphu Si was twice as short as the average size (Fig. 1B, arrows). High fruit weight was found in Mon Thong

(5.3 kg), Keng Thong (5.2 kg) and Chat Si Thong (4.0 kg) as shown in Fig. 1C. The highest fruit stalk length was found in Kan Yao Song Huat (Fig. 1D, arrows), of which the long fruit stalk was known to be the identifying character. In fact, Kan Yao in the Thai language can be translated directly as 'long fruit stalk'. The fruit spine density was in the range 6–30 spines/25 cm^2 (Fig. 1E). The highest spine density was found in Kradum Khieo (Fig. 1E, arrow). Kop Si Nak, Kop Ta Thao, Kop Si Nuan, Kop Ta Khao and Kop Champa also had high spine densities in the range 22–25 spines/25 cm^2, while Chat Si Thong, Kop Sao Noi and Mon Thong had only 6–7 spines/25 cm^2.

Mon Thong, with the heaviest fruit weight, also had an aril thickness of 2.60 cm (Fig. 1G, arrows), which was much higher than that of the other cultivars. Kop Ta Thao, Yammawat, Kop Phikun, Kop Wat Kluai and Mon Thong all had low fruit rind thickness compared to the other cultivars (Fig. 1H). A high percentage of edible flesh content by weight was found in Kop Wai, Chomphu Si, Keng Thong and Mon Thong (47%, 45%, 43% and 41%, respectively) as shown in Fig. 1I.

Pearson's correlation coefficient did not indicate any strong or remarkable associations among the quantitative traits (data not shown). However, there were significant correlations between some traits. In addition, high aril thickness also had a positive correlation with the number of immature seeds.

In this study, nine microsatellite primers were used to investigate the phylogenetic relationships of 24 cultivars. The size of the amplified bands was in the range 146–275 bp. The total number of alleles from the polymorphic loci was 21 and the allelic diversity from the nine primers was in the range 2–3 alleles per locus (Table 4). The observed heterozygosity range was 0.292–0.708 Seven loci had an observed heterozygosity higher than the expected heterozygosity. Allelic variability was also investigated using the polymorphism information content (*PIC*). The results showed that the maximum *PIC* was 0.535, which was found in MS1CT-12 and the *PIC* of all primers was higher than 0.25, which is considered moderate and indicates that the primers were reasonably informative.

Cluster analysis

Data obtained from morpho-palatability characteristics and SSR marker analysis were used for cluster analysis. Dendrograms based on these data were constructed using UPGMA.

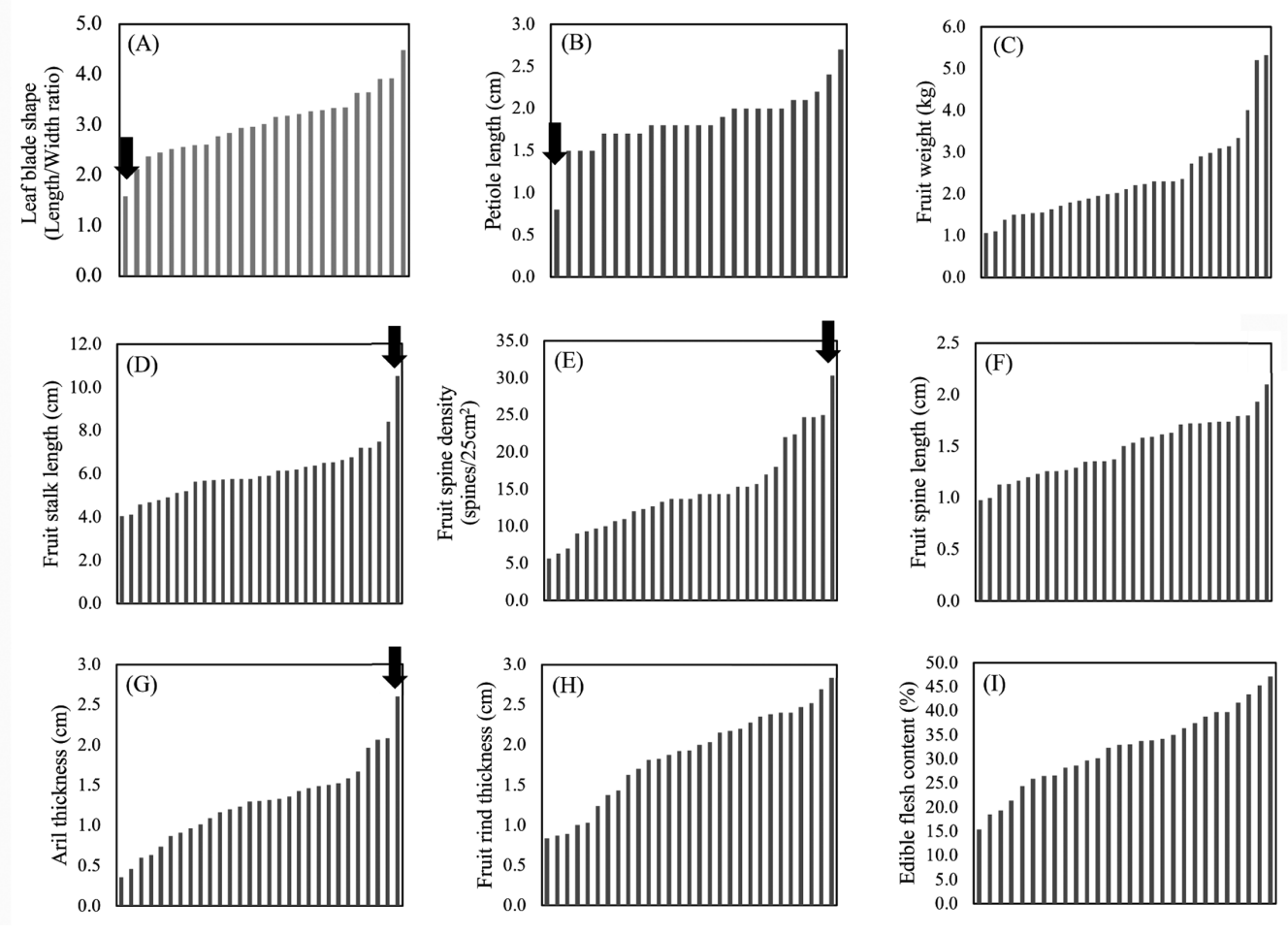

Fig. 1 Histograms of nine quantitative traits of durian cultivars from Nonthaburi province, Thailand: (A) leaf blade shape (length/width ratio), (B) petiole length (cm), (C) fruit weight (kg), (D) fruit stalk length, (E) fruit spine density, (F) fruit spine length (cm), (G) aril thickness (cm), (H) fruit rind thickness (cm), (I) edible flesh content (%); Arrows indicate noteworthy values mentioned in the text.

The dendrogram based on molecular data showed most of the 24 cultivars clustered together into two groups: A and B (Fig. 2). Kan Yao and Deang Ratsami were clustered together in clade C, while Mon Thong and Ma Fo were in clade D. Kop Chai Nam was separated from all the other cultivars in clade E. Two pairs of cultivars were indistinguishable using the nine pairs of SSR markers, namely Chomphu Si/E-Luang in clade A and Chani/Yammawat in clade B. Interestingly, these pairs shared some morphological characteristics and were previously grouped together into the Luang group (2). However, the overall clustering did not seem to correspond well with the previous grouping. The cophenetic correlation (r) of the molecular data was 0.73.

The dendrogram based on the 22 qualitative and 33 quantitative morpho-palatability characteristics showed that similar to the dendrogram based on molecular analysis, most cultivars were grouped together, with only a few cultivars separated into a different cluster, namely Mon Thong, Chao Ngo and Kop Phikun (Fig. 3). The observed cophenetic correlation (r) was rather low at 0.58. The clustering also did not seem to agree with grouping in the previous study. Based on this analysis, the two most similar cultivars were Kop Wat Kluai and Kampan Phuang. However, the raw data suggested that they could be distinguished by the blossom end area (3.08 and 0.05 cm^2, respectively) and fruit spine density (14 and 10 per $25cm^2$, respectively). Additionally, Kop Wat Kluai had a more symmetrical fruit shape, whereas Kampan Phuang had a distorted shape, similar to the anatomy of a human heart. The symmetry of fruit shape was not included in the morpho–palatability analysis.

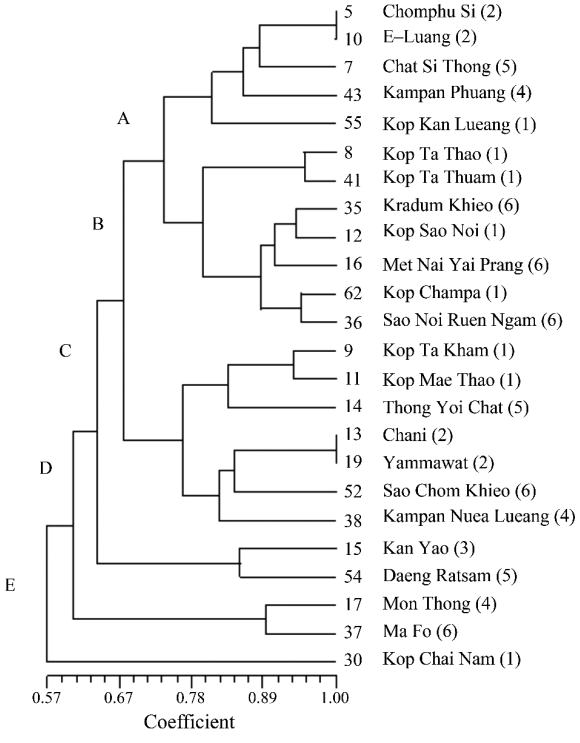

Fig. 2 Dendrogram based on molecular data using unweighted pair group method with arithmetic mean algorithm (UPGMA), where preceding numbers to cultivar names indicate accession numbers as appear in Table 1 and numbers in parentheses indicate grouping based on a previous morphological study: 1: Kop, 2: Luang, 3: Kan Yao, 4: Kampan, 5: Thong Yoi and 6: Miscellaneous (Hiranpradit et al., 1992).

Discussion

This study was the first that reported the morpho-palatability and genetic diversity of durian. Of over 200 named durian cultivars in Thailand, 42 were collected and examined. The leaves, flowers and fruits of 31 cultivars were measured for 22 qualitative and 33 quantitative characters. Furthermore, SSR primers were also used to assess genetic diversity and relatedness among 24 of the 41 cultivars. Overall, there was considerable diversity among the cultivars. The analysis of the qualitative and quantitative traits showed that some cultivars were outstanding and could be valuable resources for breeding to improve other cultivars.

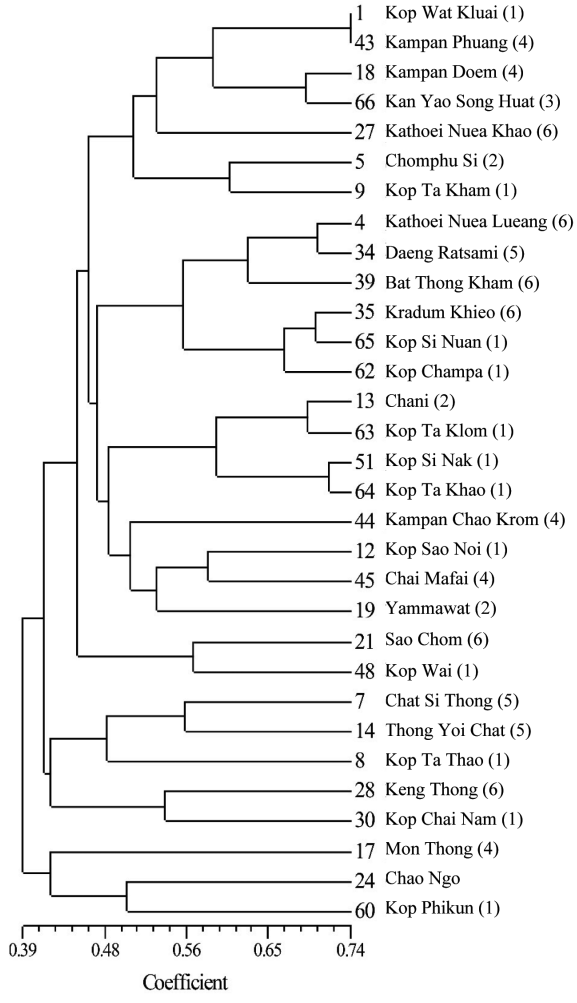

Fig. 3 Dendrogram constructed based on qualitative and quantitative morpho-palatability data using unweighted pair group method with arithmetic mean (UPGMA) algorithm, where preceding numbers to cultivar names indicate accession numbers as appear in Table 1 and numbers in parentheses indicate grouping based on a previous morphological study: 1: Kop, 2: Luang, 3: Kan Yao, 4: Kampan, 5: Thong Yoi and 6: Miscellaneous. Note: Chao Ngo was not mentioned in the previous study (Hiranpradit et al., 1992).

It was noticed by gardeners (interviewed by the authors) that the high-weight fruit often displayed a thick aril and long and sparse spines. Furthermore, some morphological durian characters were distinguishable and often used for cultivar identification, while some were important to the consumer.

The results of the morphological characterization did not correspond well with the previous studies. The discrepancy could have been due to environmental influence, misidentification or the use of different parameters for the analysis. Owing to their highly polymorphic nature, characters involving shapes (especially fruit shape and fruit apex) had high potential to use as discriminators. These parameters coincided with the characters that have been used by the orchard owners. Nonetheless, certain irregular shapes unique to some cultivars were not well-represented in the descriptor and should be improved in future work.

The color traits comprising the color of the flesh, fruit skin, seed coat and fruit stalk tended to be too polymorphic. Although the color of plant parts can be highly variable naturally, the color traits can be biased based on the researcher and can also be influenced by lighting conditions (Croft and Chen, 2017). Thus, we suggested limiting the color choices because a large number of color choices was impractical for clustering.

Palatability characteristics can also be biased depending on the experimenters, especially those involved with tasting. For accuracy, the quantitative measurement including total soluble solid (TSS), fiber content and chemical profile using high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) are recommended for the evaluation of sweetness, presence of fiber and flesh aroma, respectively in further studies.

Characters related to the leaf, including leaf size (width and length) were influenced by environmental conditions, and thus leaf shape (length/width ratio) could be more reliable. Leaf thickness and roughness of the abaxial leaf surface were also used by orchard owners for identification. However, these are difficult to measure. Generally, characters related to flowers depended on genetic factors. However, they are rarely used in folk grouping due to their limited seasonal availability.

A few characters are commonly used for grouping by orchard owners, including fruit stalk length, which can clearly separate durian cultivars in the Kan Yao group from the others. Moreover, fruit spine shape and fruit spine density can also be used for identification.

Among the most outstanding cultivars is Mon Thong, the most popular and widely cultivated durian cultivar from Thailand. Mon Thong was ranked among the top in the fruit weight, aril thickness, and edible flesh content. However, there were other, much less well–known, cultivars with even higher edible flesh contents. On the other hand, a few other cultivars had much smaller fruits, which are becoming more desirable in modern society with where a small family size is more common. A number of consumers also prefer less sweet and less pungent flesh. Therefore, conserving the genetic diversity is the key to generating more cultivars for the future.

The dendrogram based on SSR marker analysis showed that the cultivars were separated into two large clades A, and B, and three smaller clades. Interestingly, Mon Thong was placed in the smaller clade D with the Ma Fo cultivar. Although the obtained cophenetic correlation coefficient was rather low, it was comparable to that reported for *Actinidia* species and olive cultivars (Belaj et al., 2003; Korkovelos et al., 2008). Unfortunately, the genetic materials were not available for all cultivars. Therefore, the clustering based on morpho-palatability characters could not be compared to that based on the molecular data.

The previous application of ISSR markers to assess the genetic diversity of 14 durian cultivars from Nonthaburi resolved the cultivars into two major clusters (Vanijajiva, 2012). The clustering also did not correspond well with the grouping based on RAPD markers (Vanijajiva, 2011) and morphological characters (Somsri, 2007). With the availability of the next-generation sequencing, the development of markers based on whole genome sequences could prove more reliable and informative.

There were a few limitations in this study. First, not all characters were measured for all cultivars. This was due to the different timing for availability of leaves, flowers, and fruits of different cultivars. The major obstacle was the great flooding in Thailand in 2011, causing the devastating loss of most durian trees in Nonthaburi. In fact, some cultivars are known to be unrecoverable. The recent severe weather conditions and urbanization have posed a significant threat to the valuable durian genetic resource. Several persisting cultivars showing unique characters should be urgently propagated and used for breeding purpose. Though some conservation efforts have been promoted, much more research is needed to secure the future of these exotic durian cultivars.

Acknowledgements

The authors thank the Plant Genetic Conservation Project under the Royal Initiative of Her Royal Highness Princess Maha Chakri Sirindhorn for initiating the durian conservation project and all the durian orchard owners for providing research materials and informative local wisdom. This research was supported by the National Research Council of Thailand (NRCT).

References

Anderson, J.A., Churchill, G.A., Autrique, J.E., Tanksley, S.D., Sorrells, M.E. 1993. Optimizing parental selection for genetic linkage maps. Genome 36: 181–186.

Bassam, B.J., Caetano-Anollés, G., Gresshoff, P.M. 1991. Fast and sensitive silver staining of DNA in polyacrylamide gels. Anal. Biochem. 196: 80–83.

Belaj, A., Staovic, Z., Cipriani, G., Baldoni, L., Testolin, R., Rallo, L., Trujillo, I. 2003. Comparative study of the discriminative capacity of RAPD, AFLP and SSR markers and their effectiveness in establishing genetic relationships in olive. Theor. Appl. Genet. 107: 736–744.

Bioversity. 2007. Descriptors for Durian (*Durio zibethinus* Murr.). Bioversity International. Rome, Italy.

Brown, M.J. 1997. Durio - A bibliographic review. In: Arora, R.K., Ramanatha, V., Rao, A.N. (Eds). IPGRI Office for South Asia. New Delhi, India.

Croft, H., Chen, J.M. 2017. Leaf pigment content. In: Liang, S.(Ed.) Comprehensive Remote Sensing. University of Toronto, Toronto, ON, Canada. pp. 117–142

De La Loubère, S. 1986. A New Historical Relation of the Kingdom of Siam, Oxford University, Singapore, Singapore.

Ellegren, H. 2004. Microsatellites: Simple sequences with complex evolution. Nat. Rev. Genet. 5: 435–445.

Feng, Z.Y., Zhang, L.L., Zhang, Y.Z., Ling, H.Q. 2006. Genetic diversity and geographical differentiation of cultivated six-rowed naked barley landraces from the Qinghai-Tibet plateau of China detected by SSR analysis. Genet. Mol. Biol. 29: 330–338.

Hiranpradit, H., Jantrajoo, S., Lee-Ungulasatian, N., Polprasid, P. 1992. Group characterization of Thai durian, *Durio zibethinus* Murr. Acta. Hortic. 321: 263–269.

Kalinowski, S.T., Taper, M.L., Marshall, T.C. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Mol. Ecol. 16: 1099–1106.

Kittiwarodom, R., Jutamanee, K. 2011. Identification of durian cultivars using morphological characteristics and amplified fragment length polymorphism (AFLP). In: Proceedings of the 5th Botanical Conference of Thailand. Bangkok, Thailand, pp. 1–9.

Korkovelos, A.E., Mavromatis, A.G., Huang, W.G., Hagidimitriou, M., Giakoundis, A., Goulas, C.K. 2008. Effectiveness of SSR molecular markers in evaluating the phylogenetic relationships among eight Actinidia species. Sci. Hortic. 116: 305–310.

Lowe, A., Harris, S., Ashton, P. 2004. Ecological Genetics Design, Analysis, and Application. Blackwell Publishing. Oxford, UK.

Nanthachai, S. 1994. Durian: Fruit Development, Postharvest Physiology, Handling and Marketing in ASEAN. ASEAN Food Handling Bureau. Kuala Lumpur, Malaysia.

Peakall, R., Smouse, P.E. 2012. GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics 28: 2537–2539.

Rohlf, F.J. 1992. NTSYS–pc Numerical Taxonomy and Multivariate Analysis System. Exeter Software. New York, NY, USA.

Rohlf, F.J. 2000. NTSYS-pc Numerical Taxonomy and Multivariate Analysis System, Version 2.1 Exeter Software. New York, NY, USA.

Romesburg, H.C. 1984. Cluster Analysis for Researchers. Lifetime Learning Publications. Belmont, CA, USA.

Somsri, S. 2007. Thai Durian. Horticulture Research Institute, Department of Agriculture. Bangkok, Thailand.

Songnuan, W., Pichakum, A., Swangpol, S., Charoensap, P.P. 2014. Genetic resource assessment and conservation of durian (*Durio zibethinus* L.) in Thailand: Study case in Nonthaburi, Chanthaburi and Chumphon. In: Swangpol, S. (Ed.). Conservation of Durian Genetic Resources and Local Wisdoms of Durian Gardeners in Thailand for Sustainable Utilizations based on Guideline of Plant Genetic Conservation Project under the Royal Initiatives of Her Royal Highness Princess Maha Chakri Sirindhorn: Study Case in Nonthaburi, Chanthaburi and Chumphon. National Research Council of Thailand. Bangkok, Thailand. 258 pp.

SPSS Inc. 2009. PASW Statistics for Windows, Version 18.0. SPSS Inc., Chicago, IL, USA.

Subhadrabandhu, S., Ketsa, S. 2001. Durian: King of Tropical Fruit. Daphne Brasell Associates. Wellington, New Zealand.

Tessier, C., David, J., This, P., Boursiquot, J.M., Charrier, A. 1999. Optimization of the choice of molecular markers for varietal identification in *Vitis vinifera* L. Theor. Appl. Genet. 98: 171–177.

Vanijajiva, O. 2011. Genetic variability among durian (*Durio zibethinus* Murr.) cultivars in the Nonthaburi province, Thailand detected by RAPD analysis. International Journal of Agricultural Technology 7: 1105–1114.

Vanijajiva, O. 2012. The application of ISSR markers in genetic variance detection among Durian (*Durio zibethinus* Murr.) cultivars in the Nonthaburi province, Thailand. Procedia Eng. 32. 155–159. doi: 10.1016/j.proeng.2012.01.1250.

Watson, B.J. 1984. Bombacaceae. In: Page, P.E. (Ed.). Tropical Tree Fruits for Australia. Queensland Department of Primary Industries. Brisbane, Australia, pp. 45–50.