International Journal of Agricultural Technology 2024 Vol. 20(4):1497-1514
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Identification of SRAP and AFLP molecular markers associated with fruit traits in santol (*Sandoricum koetjape*)

Pedklang, N.[1], Poeaim, S.[2*], Sabpayakom, N.[2] and Vanijajiva, O.[3]

[1]Department of Science and Bioinnovation, Faculty of Liberal Arts and Science, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand; [2]Department of Biology, School of Science, King Mongkut's Institute of Technology Ladkrabang (KMITL), Ladkrabang, Bangkok 10520 Thailand; [3]Faculty of Science and Technology, Phranakhon Rajabhat University, Bangkok, 10220, Thailand.

Pedklang, N., Poeaim, S., Sabpayakom, N. and Vanijajiva, O. (2024). Identification of SRAP and AFLP molecular markers associated with fruit traits in Santol (*Sandoricum koetjape*). International Journal of Agricultural Technology 20(4):1497-1514.

Abstract The genetic diversity of santol (*Sandoricum koetjape*) using sequence-related amplified polymorphism (SRAP) and amplified fragment length polymorphism (AFLP) markers was investigated. Both molecular markers showed associations between fruit quality traits of economic importance, such as fruit weight and sweetness. The SRAP results revealed 128 amplified bands across nine primer pairs, indicating a polymorphism rate of 41.41%. Moreover, utilizing a genetic similarity coefficient of 0.85, santol cultivars were categorized into two major groups. Additionally, the AFLP analysis identified 171 amplified bands from seven primer pairs with a genetic similarity coefficient of 0.79. Consequently, santol cultivars were further classified into three distinct groups based on these findings. When combining the SRAP and AFLP results, a total of 299 amplified bands were analyzed, resulting in the separation of santol cultivars into four groups. These findings demonstrated that using SRAP and AFLP, as well as their combined results, could elucidate the genetic diversity within santol cultivars and may contribute to their classification.

Keywords: *Sandoricum koetjape*, Santol, SRAP markers, AFLP markers

Introduction

The Meliaceae, commonly called the mahogany family, are extensively distributed across tropical and subtropical regions and increasingly have extended into temperate areas. This family encompasses 740 species distributed among 58 genera (Muellner-Riehl and Rojas-Andrés, 2022). However, the Meliaceae family has limited species with edible fruit. Langsat (*Lansium domesticum*) and santol (*Sandoricum koetjape*) are notable exceptions, producing edible fruits within this family. These fruits are found in the wild and are cultivated in Southeast Asia, specifically in regions such as Thailand, Malaysia, Indonesia, and the Philippines (Yadav *et al*., 2015). In Thailand, the Meliaceae have been documented with an account covering 18 genera, encompassing 84 species, three subspecies, and four varieties (Wongprasert *et al*., 2011). Notably, this documentation introduced a novel

[*] **Corresponding Author:** Poeaim, S.; **Email:** supattra.poe@kmitl.ac.th

species, *Toona calcicole*, and a newly recorded species, *Reinwardtiodendron humile*, to the flora of Thailand (Rueangruea *et al*., 2015). Additionally, the region boasts approximately 22 edible fruit plant species that are popular among the local population. Notably, only langsat and santol are typically cultivated for commercial production.

The primary attention on santol by researchers has been directed toward its principal phytochemical components, exploring their biological activities. These constituents serve as valuable reservoirs of functionally bioactive compounds, including the flavonoids, koetjapic acid, sandoricin, sandrapins A–E, and koetjapins A–C (Ismail *et al*., 2003; 2004; Bailly, 2022; Wijaya, 2022). This plant has been subject to reviews highlighting its diverse pharmacological activities. Notably, antibacterial activity has been observed in various solvent extracts from the leaf, seed, and root of santol, as evidenced by studies from Azziz *et al*. (2013), Elijah *et al*. (2016), and Limsuwan and Voravuthikunchai (2013). Purified compounds derived from santol, including sandorinic acid A, sentulic acid, 3-oxo-olean-12-en-27-oic acid, and koetjapic acid, have demonstrated cytotoxic activities and robust anticancer properties (Tanaka *et al*., 2001; Efdi *et al*., 2012; Nassar *et al*., 2012). The extract and individual constituents of santol have been documented to exhibit antioxidant and anti-inflammatory activities (Anantachoke *et al*., 2016; Itoh *et al*., 2018).

The primary molecular approach utilized with *L. domesticum* involves random amplified polymorphic DNA (RAPD) that has revealed substantial genetic variation (Song *et al*., 2000; Nualsri *et al*., 2001; Konlasuk *et al*., 2001; Te-Chato *et al*., 2005; Yulita, 2011; Hanum *et al*., 2012). Additionally, simple sequence repeats (SSR) have been used by Efendi *et al*. (2022) and DNA barcodes have been used by Syamsuardi *et al*. (2018) to identify genetic diversity. While there is a wealth of reported genetic variations for langsat, there is a notable absence of studies focusing on the genetic diversity of santol.

Sequence-related amplified polymorphism (SRAP) and amplified fragment length polymorphism (AFLP) are polymerase chain reaction (PCR)-based techniques extensively utilized to identify polymorphisms in DNA sequences, particularly in studies of biological diversity. SRAP is designed to amplify open-reading frames (ORFs) in genomic DNA, whereas AFLP entails the selective amplification of a specific subset of genomic DNA fragments. Both SRAP and AFLP are potent techniques that do not require prior knowledge of the DNA sequence.

Despite the complexity of the procedure in AFLP, it has been highly effective and repeatable. In contrast, SRAP is characterized by simplicity, reliability, and consistent repeatability. This current study examined santol cultivars in regions where commercial cultivation occurs, specifically in Nonthaburi, Lopburi, Nakhon Nayok, and Prachinburi provinces, Thailand. The research finding aimed to analyze the genetic diversity of santol (*S.*

koetjape) using SRAP and AFLP markers to create DNA fingerprints for santol, and to evaluate the effectiveness of these methods for cultivar identification and examine their correlation with fruit quality.

Materials and methods

Plant materials

Santol fruits were gathered from various provinces in Thailand where commercial cultivation takes place, namely Nonthaburi, Lopburi, Nakhon Nayok, and Prachinburi. The collected fruits were assigned specific sample codes, cultivars, and provinces, as detailed in Table 1.

Quality of fruits

Samples were collected in June–July 2015, aligning with the harvesting season determined by growers, counting from the blooming time of santol flowers. Only fully mature fruits, neither excessively green nor overly ripe, were selected for collection. Three fruits were gathered from each tree to conduct triplicate experiments. The economic importance of santol fruit was evaluated based on parameters such as fruit weight, flesh thickness, sweetness, and pH. Fruit weight was determined in grams, and flesh thickness was measured in centimeters using a set of Vernier calipers. Sweetness was assessed by extracting juice from the seed coat and measuring it using a refractometer in Brix units (°Bx). In addition, the pH of the seed coat juice was measured using a pH meter. The collected data were categorized and analyzed using the R Studio program as an integrated development environment for R, a programming language for statistical computing and graphics (R Core Team, 2020).

DNA extraction

DNA extraction was performed using the cetyltrimethylammonium bromide (CTAB) method, following the procedure described by Subpayakom *et al*. (2016), focusing on santol DNA extraction. This method yielded high-quality DNA from leaf samples at their mature stages. Genomic DNA was purified using a GF-1 AmbiClean Kit (Gel & PCR) before DNA amplification. The quality and quantity of extracted DNA were checked based on agarose gel electrophoresis.

Table 1. Sample codes for cultivars and provinces in which fruits were collected

Sample code	Cultivar	Province	Sample code	Cultivar	Province
KT12	E-lah	Lopburi	KT34	Puifai	Nonthaburi
KT13	Thongbaiyai	Lopburi	KT36	Nimnuan	Nonthaburi
KT15	Puifai	Lopburi	KT38	E-lah	Nakhon Nayok
KT16	Puifai	Lopburi	KT39	E-lah	Nakhon Nayok
KT17	Thongkammayi	Lopburi	KT40	Thongkammayi	Nakhon Nayok
KT18	E-lah	Lopburi	KT42	Puifai	Nakhon Nayok
KT19	Thongbaiyai	Lopburi	KT43	Puifai	Nakhon Nayok
KT20	Thomtong	Lopburi	KT44	Tubtim	Nakhon Nayok
KT21	Nimnuan	Lopburi	KT45	Khanham	Nakhon Nayok
KT22	Puifai	Nonthaburi	KT46	Thongkammayi	Prachinburi
KT23	Puifai	Nonthaburi	KT47	Puifai	Prachinburi
KT26	Tubtim	Nonthaburi	KT48	Keawnumpueng	Prachinburi
KT27	Khanthong	Nonthaburi	KT49	Puifai	Prachinburi
KT28	Khiaowan	Nonthaburi	KT50	Tubtim	Prachinburi
KT29	Tubtim	Nonthaburi	KT51	Tubtim	Prachinburi
KT30	Tubtim	Nonthaburi	KT52	Khiaowan	Prachinburi
KT31	Puifai	Nonthaburi	KT54	Puifai	Nakhon Nayok
KT33	Puifai	Nonthaburi	KT55	Wild santol	Nakhon Nayok

Sequence-related amplified polymorphism (SRAP) markers

At the outset, a set of 30 combinations, consisting of five forward primers (ME1–ME5) and six reverse primers (EM1–EM6), as outlined in Table 2, were screened in the Puifai (KT22) and Khiaowan (KT28) cultivars. The PCR reaction was conducted in a final volume of 20 µL, comprising 100 ng of high-quality genomic DNA, 0.8 µM each primer, 0.20 mM dNTPs mix, 2.5 mM $MgCl_2$, 1U of *Taq* DNA polymerase, and 1XPCR buffer. The experimental procedures followed the protocols established by Subpayakom *et al*. (2016). The PCR amplification program consisted of an initial denaturation at 94°C for 3 minutes, followed by five cycles of denaturation at 94°C for 1 minute, annealing at 35°C for 1 minute, and elongation at 72°C for 1 minute. Subsequently, 35 cycles were performed with denaturation at 94°C for 1 minute, annealing at 50°C for 1 minute, and elongation at 72°C for 1 minute. The final step involves one extension at 72°C for 10 minutes. The amplified SRAP fragments were separated on a 2% agarose gel stained in 1XTBE buffer with ethidium bromide to establish the SRAP fragment

compared with a 100 base pairs DNA ladder (Vivantis) and made into an SRAP profile.

Table 2. Sequences of five forward and six reverse primers

Primer		Sequences (5' to 3')	Primer		Sequences (5' to 3')
Forward primer	Me1	TGAGTCCAAACCGGATA	Reverse primer	Em1	GACTGCGTACGAATTAAT
	Me2	TGAGTCCAAACCGGAGC		Em2	GACTGCGTACGAATTTGC
	Me3	TGAGTCCAAACCGGAAT		Em3	GACTGCGTACGAATTGAC
	Me4	TGAGTCCAAACCGGACC		Em4	GACTGCGTACGAATTTGA
	Me5	TGAGTCCAAACCGGAAG		Em5	GACTGCGTACGAATTAAC
				Em6	GACTGCGTACGAATTGCA

Amplified fragment length polymorphism (AFLP) markers

The DNA sample underwent double digestion with *Eco*RI and *Mse*I enzymes at 37°C overnight. Subsequently, the digested DNA was ligated to *Eco*RI and *Mse*I adapters in T4 ligase buffer at 37°C for 3 hours. The preselected amplification step used primers complementary to the adapters, each containing one additional nucleotide (*Eco*RI+A and *Mse*I+C), as specified in Table 3. Then, the selective PCR amplification step was performed using three selective nucleotides (*Eco*RI+ANN and *Mse*I+CNN), as shown in Table 3. In total, 40 pairs of *Eco*RI and *Mse*I primers were used to screen four samples of santol: Puifai (KT22), Khanthong (KT27), Khiaowan (KT28), and Thongkammayi (KT40). The PCR product was mixed with an equal volume of AFLP loading dye, comprising 98% formamide, 10 mM EDTA (pH 8.0), 0.1% bromophenol blue, and 0.1% xylene cyanol. For denaturation, the samples were heated at 90°C for 5 minutes and then placed on ice. Subsequently, the amplified DNA fragments from each sample and primer were separated using a denaturing 6% polyacrylamide gel.

Statistical analysis

All experimental measurements were performed in triplicate and expressed as mean ± standard deviation (SD) values. Data analysis was performed using the Statistical Package for the Social Sciences version 17.0.

Results

Study of santol fruit quality for economic importance

Data regarding the economic importance of santol fruit quality are shown in Table 4. Puifai (KT47) registered the maximum weight (758.33 g), whereas Tubtim (KT26) had the lowest weight (125 g). Puifai (KT33) was the sweetest fruit (21°Bx). E-lah (KT12) produced the lowest pH (2.77)

among the fruits. The dendrogram representing santol fruit weight (Figure 1A) revealed that Puifai, E-lah, and Khanham fell into the category of heavy fruits (weight range, 325.00–758.33 g). On the other hand, Tubtim, Thongkammayi, Nimnuan, Thongbaiyai, Khiaowan, and Khanthong were identified as small santol fruits (weight range, 125.00–298.33 g). The sweetness dendrogram indicated that Puifai (KT22, KT31, KT33, and KT43), Nimnuan (KT36), Tubtim (KT26), E-lah (KT38 and KT39), Keawnumpueng (KT48), Khiaowan (KT28), Thongkammayi (KT40), Khanthong (KT27), Thongbaiyai (KT13), and Thomtong (KT20) were categorized in the highly sweet group, displaying sweetness levels in the range 17.53–21°Bx, while Thongkammayi (KT17 and KT46), Tubtim (KT29 and KT30), Puifai (KT15, KT23, KT34, and KT47), Nimnuan (KT21), E-lah (KT12 and KT18), and Khanham (KT45) fell into the less sweet group, with sweetness levels in the range 11.6–16.2°Bx, as illustrated in Figure 1B.

Table 3. Sequences of adapter and primers in AFLP markers

Adaptors and primers	**Sequences (5' to 3')**
Adaptor *Eco*RI A1	CTCGTAGACTGCGTACC
Adaptor *Eco*RI A2	CATCTGACGCATGGTTAA
Adaptor *Mse*I A1	GACGATGAGTCCTGAG
Adaptor *Mse*I A2	TACTCAGGACTCAT
*Eco*RI-A	GACTGCGTACCAATTCA
*Eco*RI-AAC	GACTGCGTACCAATTCAAC
*Eco*RI-AAG	GACTGCGTACCAATTCAAG
*Eco*RI-ACA	GACTGCGTACCAATTCACA
*Eco*RI-ACC	GACTGCGTACCAATTCACC
*Eco*RI-ACG	GACTGCGTACCAATTCACG
*Eco*RI-ACT	GACTGCGTACCAATTCACT
*Eco*RI-AGA	GACTGCGTACCAATTCAGA
*Eco*RI-AGC	GACTGCGTACCAATTCAGC
*Eco*RI-AGG	GACTGCGTACCAATTCAGG
*Eco*RI-AGT	GACTGCGTACCAATTCAGT
*Mse*I-C	GATGAGTCCTGAGTAAC
*Mse*I-CAA	GATGAGTCCTGAGTAACAA
*Mse*I-CAC	GATGAGTCCTGAGTAACAC
*Mse*I-CAG	GATGAGTCCTGAGTAACAG
*Mse*I-CAT	GATGAGTCCTGAGTAACAT

Based on the experiment, samples from the cultivars Puifai, Nimnuan, Tubtim, E-lah, and Thongkammayi were distributed across both the sweet and less-sweet groups. Similarly, the pH testing results indicated variation within a single cultivar, with fruits exhibiting high pH levels (pH 2.98–3.38) and low pH levels (pH 2.77–2.90). These results suggested that sweetness and pH alone may not be reliable identifiers for santol cultivars. The variability observed in these attributes within cultivars may indicate external factors influencing fruit characteristics, such as fertilization, climate, soil conditions, and other environmental factors.

Table 4. Characteristics of economic importance

Santol	Weight (g)	Flesh size (cm)	Sweetness (°Bx)	Flesh pH
KT12 E-lah	368.33	13.58	15.2	2.77
KT13 Thongbaiyai	290.00	12.83	18.00	3.15
KT15 Puifai	216.67	11.76	16.00	3.13
KT17 Thongkammayi	205.00	11.10	16.20	3.13
KT18 E-lah	365.00	13.77	14.53	3.10
KT20 Thomtong	296.67	12.94	18.13	3.12
KT21 Nimnuan	396.67	12.68	14.27	3.29
KT22 Puifai	351.67	14.90	18.80	2.90
KT23 Puifai	298.33	11.30	15.73	3.05
KT26 Tubtim	125.00	9.47	18.87	2.98
KT27 Khanthong	263.33	14.65	17.87	3.01
KT28 Khiaowan	288.33	12.24	17.53	2.99
KT29 Tubtim	380.00	15.52	14.50	3.17
KT30 Tubtim	275.00	13.60	15.53	3.23
KT31 Puifai	388.33	14.56	20.17	3.17
KT33 Puifai	408.33	15.01	21.00	2.83
KT34 Puifai	368.33	16.93	15.93	3.02
KT36 Nimnuan	296.67	13.67	19.20	3.15
KT38 E-lah	466.67	17.67	17.67	3.38
KT39 E-lah	448.33	17.07	18.13	3.07
KT40 Thongkammayi	236.67	13.31	18.33	3.29
KT42 Puifai	340.00	13.35	20.07	3.01
KT43 Puifai	460.00	20.10	17.53	3.18
KT45 Khanham	325.00	12.74	14.93	2.79
KT46 Thongkammayi	265.00	13.59	11.60	3.27
KT47 Puifai	758.33	20.33	15.27	3.27
KT48 Keawnumpueng	476.67	12.89	17.54	2.85

Note: Fruit samples of KT16, KT19, KT44, KT49, KT50, KT51, KT52, KT54, and KT55 could not be collected because of the absence of fruit on these trees.

SRAP analysis

Initially, SRAP primer combination sets were used to develop marker profiles for two different morphologies: Puifai (KT22) with large fruit and Khiaowan (KT28) with small fruit. Nine primer pairs that exhibited reproducible fragments with easily recordable bands and demonstrated polymorphisms are presented in Table 5. In the initial phase, primer combinations were used to create marker profiles for two distinct morphologies: Puifai (KT22), featuring large fruits, and Khiaowan (KT28), characterized by small fruits. Information on the nine primer pairs (number of amplified bands, polymorphic bands, and polymorphism percentage) is displayed in Table 5.

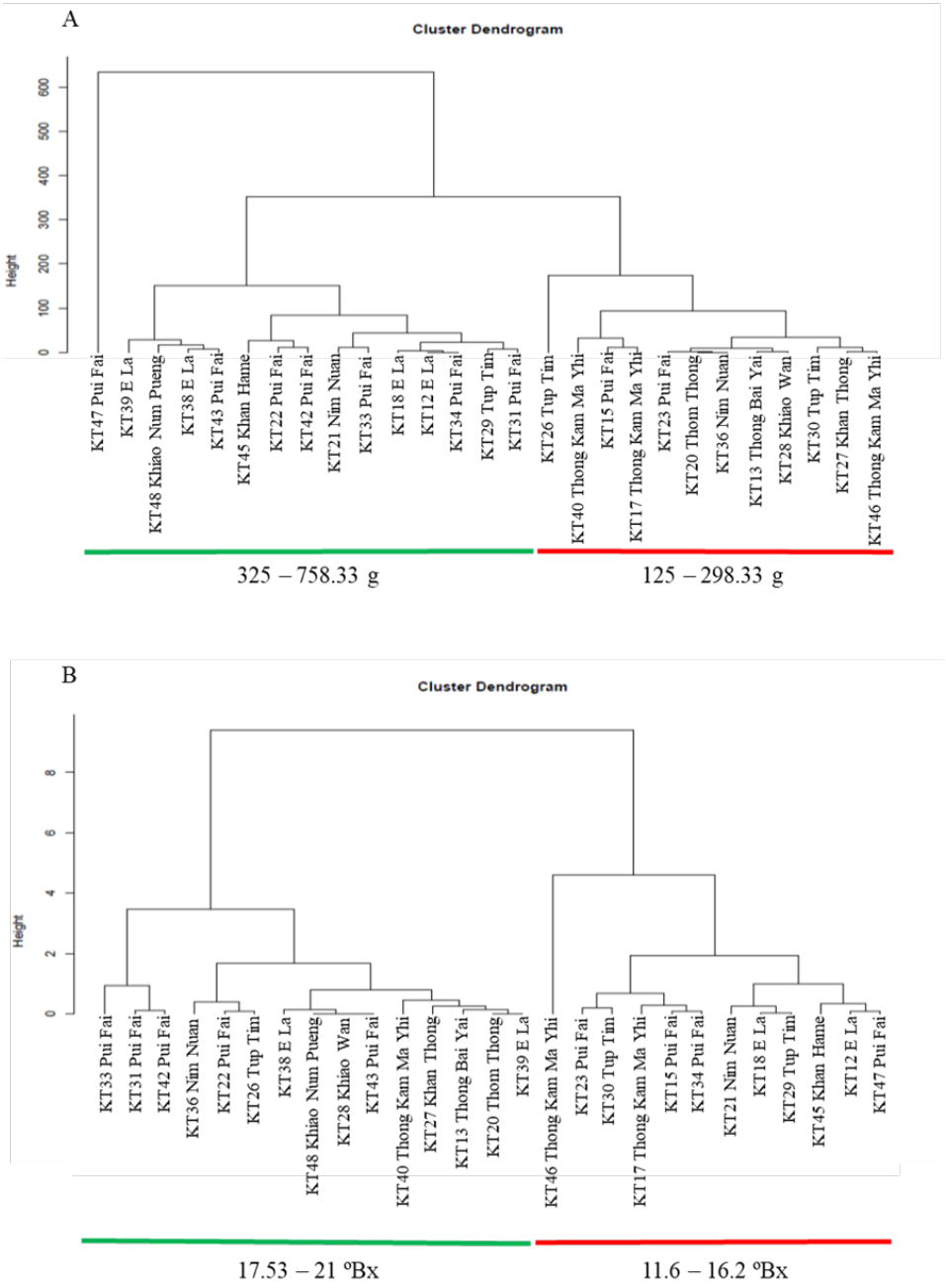

Figure 1. Dendrograms of Santol economic importance: (A) fruit weight and (B) fruit sweetness

Table 5. Number of amplified bands, polymorphic bands, and percentage polymorphism of nine selected SRAP primer pairs

Primer pair	Number of amplified bands	Number of polymorphic bands	Percentage polymorphism
Me1/Em3	15	4	26.67
Me1/Em5	11	3	27.27
Me2/Em5	13	4	30.77
Me2/Em6	12	4	33.33
Me3/Em3	15	6	40.00
Me3/Em4	16	5	31.25
Me4/Em1	13	8	61.54
Me5/Em2	17	10	58.82
Me5/Em4	16	9	56.25
Total	128	53	
Mean	14.22	5.89	41.41

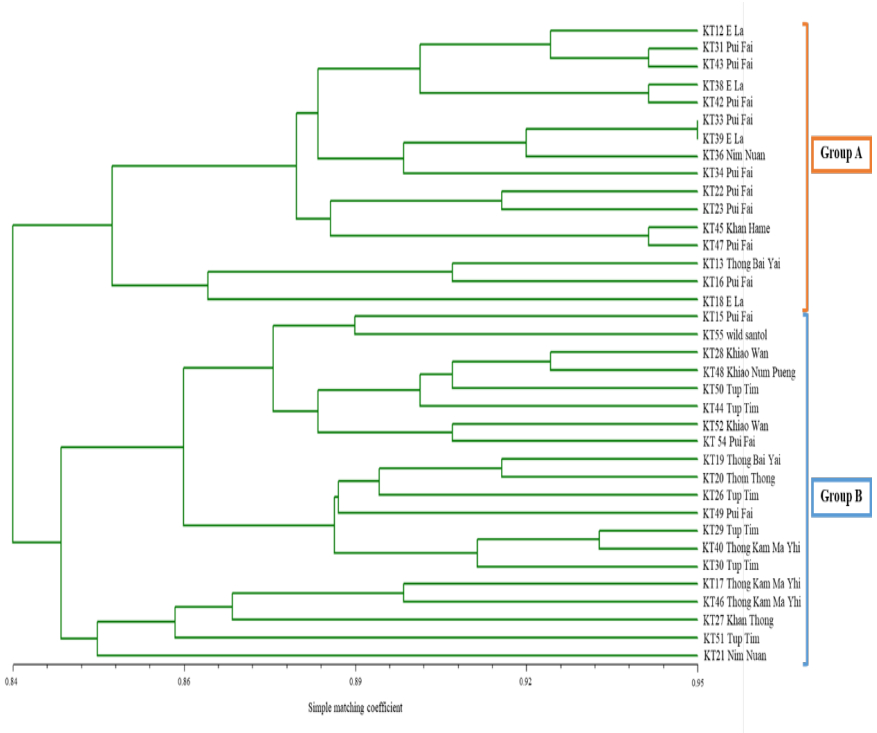

Figure 2. Dendrogram of 36 santol samples was analyzed based on SRAP markers using NTsyspc version 2.11X program and UPGMA method

All santol DNA samples were analyzed using a simple matching coefficient from the nine selected primer pairs using NTsyspc version 2.11X. The genetic similarity coefficient was in the range of 0.76–0.95, with an average of 0.85. The genetic similarity coefficient for the dendrogram constructed using the UPGMA method was 0.84, with the classification being

into two major groups, as illustrated in Figure 2. Group 1 comprised Puifai (KT16, KT22, KT23, KT31, KT34, KT37, KT42, KT43, and KT47), E-lah (KT12, KT18, KT38, and KT39), Nimnuan (KT36), Khanham (KT45), and Thongbaiyai (KT13). Group 2 consisted of Puifai (KT15, KT49, and KT54), wild santol (KT55), Khiaowan (KT28 and KT52), Keawnumpueng (KT48), Tubtim (KT26, KT29, KT30, KT44, KT50, and KT51), Thongbaiyai (KT19), Thomtong (KT20), Thongkammayi (KT40, KT17, and KT46), Khanthong (KT27), and Nimnuan (KT21). However, Puifai (KT15, KT49, and KT54) was distributed across both groups, along with Nimnuan (KT21 and KT36), and Thongbaiyai (KT13 and KT19).

AFLP analysis

Variations observed in different branches of the SRAP dendrogram for Puifai (KT22), Khanthong (KT27), Khiaowan (KT28), and Thongkammayi (KT40) were used to screen the AFLP primers. The AFLP primers were used for screening in these four santol samples (Table 3). Seven primer pairs: MseI-CAA/EcoRI-ACA, MseI-CAA/EcoRI-ACC, MseI-CAA/EcoRI-ACT, MseI-CAA/EcoRI-AGC, MseI-CAA/EcoRI-AGG, MseI-CAC/EcoRI-AAG, and MseI-CAC/EcoRI-ACA produced clear and distinct DNA bands, exhibiting variations. Consequently, these seven selected primer pairs were used to study the genetic diversity of all 36 santol samples.

Through the AFLP markers, analysis of 171 DNA bands from the seven primer pairs of the 36 santol samples revealed a similarity coefficient in the range of 0.73–1.00, with an average value of 0.86.

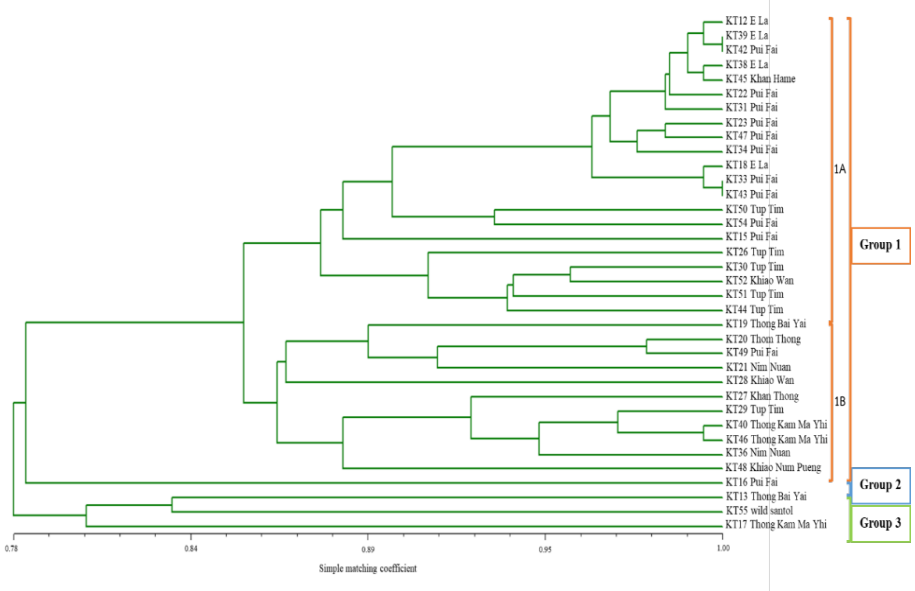

Figure 3. Dendrogram of 36 santol samples was analyzed based on AFLP markers using NTsyspc version 2.11X program and UPGMA method

The dendrogram of the 36 samples constructed using the UPGMA method classified the santol samples into three major groups with a similarity coefficient of 0.79. Group 1 comprised almost all samples (32 samples) with various cultivars. Group 2 contained Puifai (KT 16), and group 3 comprised Thongbaiyai (KT13), wild santol (KT55), and Thongkammayi (KT17), as depicted in Figure 3. This outcome suggested that KT13 and KT17 were closely related to wild santol (KT55). Group 1 could be further classified into two subgroups. Group 1A consisted of E-lah (KT12, KT38, KT39, and KT18), Puifai (KT15, KT22, KT23, KT31, KT33, KT34, KT42, KT43, KT47, and KT54), Khanham (KT45), Tubtim (KT26, KT30, KT44, KT50, and KT51), and Khiaowan (KT52). Group 1B consisted of Thongbaiyai (KT19), Thom Tong (KT49), Nimnuan (KT21 and KT36), Khiaowan (KT28), Khanthong (KT27), Tubtim (KT29), Thongkammayi (KT40, KT46), and Khiao Num Pueng (KT48).

Genetic diversity of santol using a combination of SRAP and AFLP markers

The 128 DNA-amplified bands from 9 primer pairs of 36 santol fruit samples of SRAP markers were combined with the 171 DNA-amplified bands from 7 primer pairs of 36 santol fruit samples of AFLP markers, and the resultant 299 DNA-amplified bands were analyzed based on simple matching using the NTsyspc version 2.11X program. The results showed that the similarity coefficient was in the range of 0.76–0.97, averaging 0.86. Constructing a dendrogram of the 36 samples using the UPGMA method, with a similarity coefficient of 0.82, the santol samples could be classified into four groups (Figure 4). Group 1 consisted of almost all samples, group 2 consisted of Thongbaiyai (KT13) and Thongkammayi (KT17), group 3 consisted of Puifai (KT16), and group 4 consisted of wild santol (KT55).

Group 1 could be classified further into two subgroups: group 1A consisting of E-lah (KT12, KT38, KT39 and KT18), Puifai (KT22, KT23, KT31, KT33, KT34, KT42, KT43, and KT47) and Khanham (KT45), which are large-sized fruit; and group 1B consisting of Puifai (KT15, KT49, and KT54), Khiaowan (KT28 and KT52), Khiao Num Pueng (KT48), Tubtim (KT29, KT29, KT30, KT44, KT50, and KT51), Thom Thong (KT20), Thongkammayi (KT40 and KT46), Nimnuan (KT21 and KT36), Khanthong (KT27), and Thongbaiyai (KT19), which are small-sized fruit.

Because group 1B consisted of various santol cultivars of mostly small-sized fruit, samples were clustered from the Khiaowan and Tubtim cultivars. This finding reflected the relationship between these two cultivars. In addition, the names containing ‘thong’ were similar to findings from the dendrogram of the AFLP markers.

The santol samples could be classified into 2 groups based on combining the DNA-amplified bands from the SRAP markers with the AFLP markers,

based on the distribution chart produced by the Past version 3.14 program. Group 1 consisted of Puifai, E-lah, and Khanham, which are large-sized fruits. Group 2 consisted of various cultivars of small-sized fruits, with Thongbaiyai (KT13), Puifai (KT16), Thongkammayi (KT17), and wild santol (KT55) deviating from the group. This distribution chart showed that the classification of santol was closed to the dendrogram and the distribution chart of the AFLP markers.

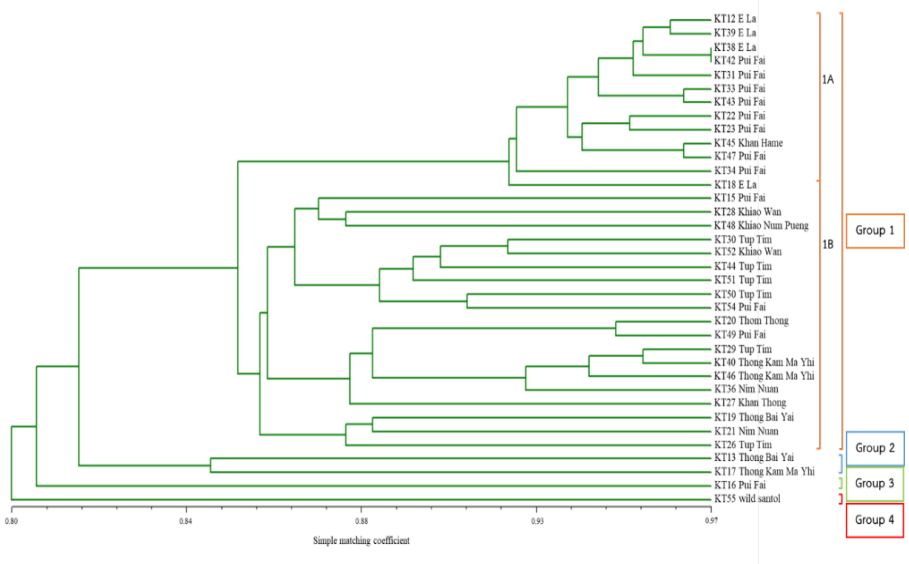

Figure 4. A dendrogram of 36 samples of santol was separated into four groups using SRAP incorporated with AFLP markers

Analysis of the genetic structure

The genetic structures derived from SRAP, AFLP, and the combination of SRAP and AFLP were analyzed using the Structure version 2.3.4 program and are illustrated in Figure 5. The three techniques produced consistent results revealing that santol genetic structures could be categorized into two distinct patterns (K=2), denoted as C1 and C2. The majority of C1 (green) varieties comprised Thongkammayi, Thongbaiyai, Thom Thong, Nimnuan, Tubtim, Khanthong, Khiaowan, Khiao Num Pueng, and wild santol, all characterized by small fruits. On the other hand, C2 (yellow) contained E-lah, Puifai, and Khanham, which are associated with larger-sized fruit.

These results are also corresponded to the classification using each technique's dendrogram and distribution chart. However, the genetic structure of santol based on SRAP had Puifai (KT15, KT49 and KT54), which are large fruits, through their genetic structure was in C1. This could have been a deviation or an error in the identification of cultivars since the initial growth of the santol plant. Furthermore, it was observed that KT13 and KT36, identified as Thongbaiyai and Nimnuan, respectively, were placed in C2.

These results suggested that the SRAP technique faced challenges in distinguishing Thongbaiyai and Nimnuan from the group of large fruits, emphasizing limitations in the ability of SRAP to differentiate these specific cultivars within the larger classification of santol genetic structures.

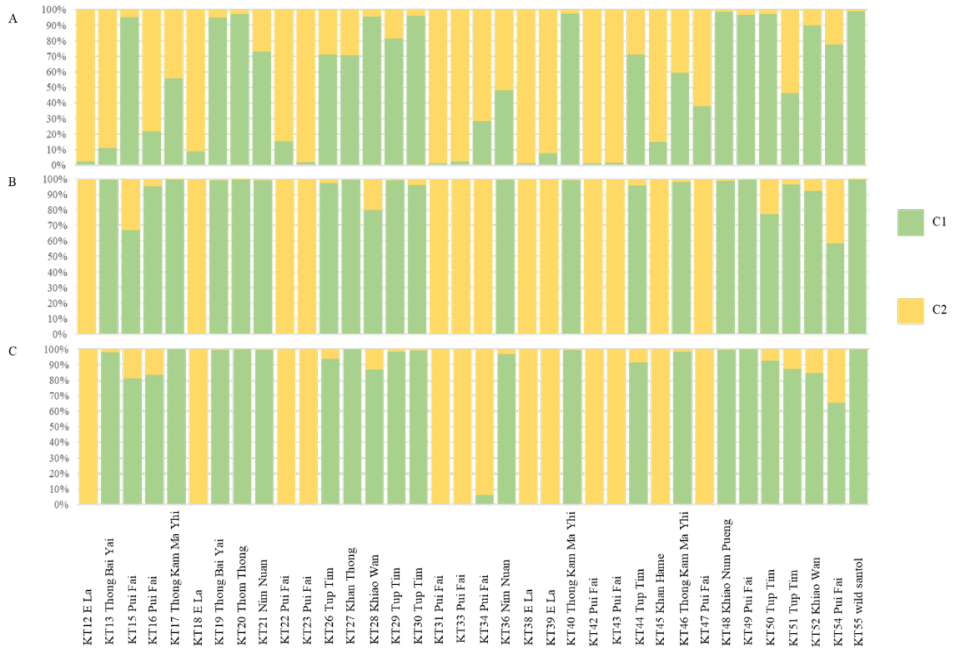

Figure 5. Genetic structure of santol from SRAP, AFLP, and combination of SRAP and AFLP techniques (C1: small-sized fruit and C2: large-sized fruit)

Discussion

Molecular marker techniques have been employed to assess the genetic diversity within plant populations. These markers can be obtained from plants at any growth stage and are unaffected by environmental conditions. SRAP markers, a PCR-based marker system, are used to amplify coding regions of DNA with primers targeting ORFs (Li and Quiros, 2001). Recently, SRAP has been successfully utilized to assess genetic diversity and construct genetic maps of various plant species (Huang *et al*., 2014; Polat *et al*., 2012). This current study demonstrated the genetic diversity of santol by SRAP markers. The dendrogram used to study santol fruit quality for economic importance showed that santol could be classified into two groups: a large fruit group, consisting of Puifai, E-lah and Khanham, and a small fruit group, consisting of Tubtim, Thongkammayi, Nimnuan, Thongbaiyai, Khiaowan, and Khanthong. Based on the dendrogram, the samples in group 1 had large fruits, whereas those in group 2 had small fruits. Combined with the dendrogram results based on santol fruit weight, the SRAP technique classification corresponded well to the santol fruit weight. Therefore, the SRAP technique

tended to accurately classify santol based on the fruit weight of the santol cultivar.

AFLP is another molecular marker used to study genetic diversity among santol species. This technique is effective and powerful compared to other methods, such as restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD). AFLP can detect various genomic regions, allowing the differentiation of closely related species, and it produces many reproducible amplified products. (Costa *et al*., 2016). For the AFLP markers, group 1A consisted of E-lah, Puifai and Khanham, which are large fruit cultivars, and the cluster of Tubtim and Khiaowan, which are small fruit cultivars. In group 1B, the names of the cultivars consisted of the word 'thong'. This could reflect that the santol fruits of these cultivars are different in color from other santol cultivars. However, color information was not collected for inclusion in this study. Despite the effectiveness of santol classifications using SRAP and AFLP techniques individually, the genetic structures obtained through the combination of SRAP and AFLP were similar to AFLP than to SRAP. This suggested that the combined approach may produce a closer resemblance to the genetic patterns revealed by AFLP, indicating a potential synergy or dominance of AFLP characteristics in the combined analysis.

SRAP and AFLP were effective in classifying santol. However, notably, the dendrogram and the distribution chart generated by AFLP could successfully distinguish wild santol from other cultivars. Additionally, they could separate Thongbaiyai and Nimnuan, both of which are small fruit santol varieties, from the larger fruit santol varieties. This indicated that AFLP had high discriminatory power in capturing genetic variations and distinctions among different santol cultivars.

The observations in the current study were consistent with those reported by Ammar *et al*. (2015) on the genetic diversity of broad bean (*Vicia faba* L.) using SRAP and AFLP markers. Their research compared the effectiveness of both markers and noted different outcomes from each technique. Notably, when they examined the dendrogram generated from the combination of SRAP and AFLP, they found that the dendrogram from AFLP was more similar to that from the combination of SRAP and AFLP, as opposed to the dendrogram from SRAP alone. This similarity emphasized the potential synergies and complementary nature of using a combined approach to capture genetic diversity compared with individual techniques.

The classifications of santol based on SRAP, AFLP, and the combined approach of both markers aligned effectively with the categorization based on fruit weight. These results suggested that santol could be reliably classified using its fruit weight. Nevertheless, evaluating fruit quality, including parameters such as fruit flesh thickness, sweetness, and pH, was inconsistent with the outcomes obtained from the three techniques mentioned above.

Diversity studies using SRAP and AFLP demonstrated the effectiveness of both techniques in classifying santol into two major groups. These groups were characterized as a large fruit group, containing varieties such as Puifai, E-lah, and Khanham, and a small fruit group consisting of Tubtim, Thongkammayi, Nimnuan, Thongbaiyai, Khiaowan, and Khanthong. This classification based on fruit size highlighted the ability of SRAP and AFLP to discern distinct patterns within the genetic diversity of santol, facilitating the categorization of cultivars into meaningful groups.

The AFLP technique demonstrated its capability to effectively classify wild santol and closely relate it to other cultivars. Based on examining genetic variance using both SRAP and AFLP, the study revealed that classifying samples by planting areas across the four provinces did not substantially impact genetic diversity. However, diversity was influenced by the various cultivars within a specific province (planting area). Despite the variability within cultivars, F_{ST} analysis indicated that santol exhibited low genetic diversity (data not shown). This suggested that although there may be differences among cultivars, the overall genetic diversity of santol as a species is comparatively low.

These results also corresponded to the classification using each technique's dendrogram and distribution chart. However, the genetic structure of santol based on SRAP analysis classified Puifai (KT15, KT49, and KT54), which are large fruits, in the C1 genetic structure. This could have been a deviation or due to an error in the identification of cultivars since the initial growth of the santol plant. Furthermore, it was observed that KT13 and KT36, identified as Thongbaiyai and Nimnuan, respectively, were placed in C2. These results suggested that the SRAP technique faced challenges in distinguishing Thongbaiyai and Nimnuan from the group of large fruits, emphasizing limitations in the ability of SRAP to differentiate these specific cultivars within the larger classification of santol genetic structures. For example, a genus such as *Cedrela balansae* C. DC. in the Meliaceae (the same family as santol), in Northwestern Argentina was assessed using a combination of SSR and AFLP molecular markers (Soldati *et al*., 2013).

In conclusion, molecular studies using SRAP and AFLP techniques effectively evaluated genetic diversity in santol. The results obtained facilitated the accurate classification of santol cultivars. The combined use of both techniques enhanced the overall effectiveness of the classification. Consequently, this research should serve as a foundational study for further exploring the relationships and the correct identification of santol cultivars. The insights gained from this study should inform plans to improve santol cultivars, potentially enhancing the economic value in the future.

Acknowledgements

This research was financially supported by the Biodiversity-Based Economy Development Office (Public Organization) (BEDO), Thailand.

References

Ammar, M. H., Alghamdi, S. S., Migdadi, H. M., Khan, M. A., El-Harty, E. H. and Al Faifi, S. A. (2015). Assessment of genetic diversity among faba bean genotypes using agro-morphological and molecular markers. Saudi Journal of Biological Sciences, 22:340-350.

Anantachoke, N., Lomarat, P., Praserttirachai, W., Khammanit, R. and Mangmool, S. (2016). Thai fruits exhibit antioxidant activity and induction of antioxidant enzymes in HEK-293 cells. Evidence-Based Complementary and Alternative Medicine, 14:6083136.

Azziz, S. S. A., Alimon, H., Abdullah Sani, A., Daud, N. and Noor, N. (2013). Phytochemical screening and antimicrobial activities of seed extracts from *Sandoricum Koetjape*. The Open Conference Proceedings Journal, 4:104.

Bailly, C. (2022). The health benefits of santol fruits and bioactive products isolated from *Sandoricum koetjape* Merr.: A scoping review. Journal of Food Biochemistry, 46: e14152.

Costa, R., Pereira, G., Garrido, I., Tavares-de-Sousa, M. M. and Espinosa, F. (2016). Comparison of RAPD, ISSR, and AFLP molecular markers to reveal and classify orchardgrass (*Dactylis glomerata* L.) germplasm variations. PLoS One, 11:e0152972.

Efendi, D., Sar, HP., Suwarno, W. B. and Matra, D. D. (2022). Genetic diversity of *Lansium parasiticum* (Osbeck) K. C. Sahni & Bennet accessions based on vegetative morphological characters and simple sequence repeat markers. Genetic Resources and Crop Evolution, 69:1707-1716.

Efdi, M., Ninomiya, M., Suryani, E., Tanaka, K., Ibrahim, S., Watanabe, K. and Koketsu, M. (2012). Sentulic acid: a cytotoxic ring A-seco triterpenoid from *Sandoricum koetjape* Merr. Bioorg Med Chem Lett, 22:4242-4245.

Elijah A, O., C Onwuchekwa, E. and Ekeleme, U. (2016). Phytochemical constituents and antimicrobial activity of *Sandoricum koetjape* leaf and seed extracts on clinical isolates from patients. Unique Research Journal of Medicine and Medical Sciences, 4:69-76.

Hanum, L., Kasiamdari, RS., Santosa, S. and Rugayah, R. (2012) Genetic relatedness among Duku, Kokosan, and Pisitan in Indonesia based on random amplified polymorphic DNA markers. Indonesian Journal of Biotechnology, 17:121-131.

Huang, C., Liu, G., Bai, C. and Wang, W. (2014). Genetic analysis of 430 Chinese Cynodon dactylon accessions using sequence-related amplified polymorphism markers. International Journal of Molecular Sciences, 15:19134-19146.

Ismail, I. S., Ito, H., Hatano, T., Taniguchi, S. and Yoshida, T. (2003). Modified limonoids from the leaves of *Sandoricum koetjape*. Phytochemistry, 64:1345-1349.

Ismail, I. S., Ito, H., Hatano, T., Taniguchi, S. and Yoshida, T. (2004). Two new analogues of trijugin-type limonoids from the leaves of *Sandoricum koetjape*. Chemical & Pharmaceutical Bulletin (Tokyo), 52:1145-1147.

Itoh, T., Katsurayama, K., Efdi, M., Ninomiya, M. and Koketsu, M. (2018). Sentulic acid isolated from *Sandoricum koetjape* Merr attenuates lipopolysaccharide and interferon gamma costimulated nitric oxide production in murine macrophage RAW264 cells. Bioorganic & Medicinal Chemistry Letters, 28:3496-3501.

Konlasuk, S., Nualsri, C. and Te-chato, S. (2001). Establishment of experimental conditions on random amplified polymorphic DNA (RAPD) analysis *Lansium domesticum* Corr. II. primer screening and identifi cation of longkong, langsat, and duku. Songklanakarin Journal of Science and Technology, 23:325-334.

Li, G. and Quiros, C. (2001). Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in Brassica. Theor Appl Genet, 103:455-461.

Limsuwan, S. and Voravuthikunchai, S. (2013). Anti-*Streptococcus pyogenes* activity of selected medicinal plant extracts used in Thai traditional medicine. Tropical Journal of Pharmaceutical Research, 12:535-540.

Muellner-Riehl, A. N. and Rojas-Andrés, B. M. (2022). Biogeography of neotropical Meliaceae: geological connections, fossil and molecular evidence revisited. Brazilian Journal of Botany, 45:527-543.

Nassar, Z. D., Aisha, A. F., Idris, N., Khadeer Ahamed, M. B., Ismail, Z., Abu-Salah, K. M. and Shah Abdul Majid, A. M. (2012). Koetjapic acid, a natural triterpenoid, induces apoptosis in colon cancer cells. Oncology Reports, 27:727-733.

Nualsri, C., Te-chato, S., Lim, M. and Chooruk, U. (2001). A survey of genetic viability of longkong (*Lansium domesticum* Corr.) seedings by RAPD (Random Amplified Polymorphic DNA) technique. Proceedings of The 39th Kasetsart University Annual Conference, 39:532-539.

Polat, I., Kacar, Y.A., Yesiloglu, T., Uzun, A., Tuzcu, O., Gulsen, O., Incesu, M., Kafa, G.,nTurgutoglu, E. and Anil, S. (2012). Molecular characterization of sour orange (*Citrus aurantium*) accessions and their relatives using SSR and SRAP markers. Genetics and Molecular Research, 11:3267-3276.

R Core Team. (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Polat, I., Kacar

Rueangruea, S., Tagane, S., Sudee, S., Tetsana, N., Poopath, M., Nagamasu, H. and Naiki, A. (2015). Toona calcicola, a new species and Reinwardtiodendron humile, a new record for Thailand. Thai Forest Bulletin (Botany), 43:79-86.

Soldati, M. C., Fornes, L., Van Zonneveld, M., Thomas, E. and Zelener, N. (2013). An assessment of the genetic diversity of *Cedrela balansae* C. DC. (Meliaceae) in Northwestern Argentina by means of combined use of SSR and AFLP molecular markers. Biochemical Systematics and Ecology, 47:45-55.

Song, B. K., Clyde, M. M., Wickneswari, R. and Normah, M. N. (2000). Genetic relatedness among *Lansium domesticum* accessions using RAPD markers. Annals of Botany, 86: 299-307.

Subpayakom, N., Poeaim, A., Vanijajiva, O. and Poeaim, S. (2016). An efficient protocol for genomic DNA extraction from santol (*Sandoricum koetjape*) for SRAP marker analysis. International Journal of Agricultural Technology, 12:1475-1482.

Syamsuardi, S., Chairul, C. and Murni, P. (2018). Analysis of genetic impurity of an original cultivar duku (Lansium parasiticum (Osbeck.) K.C. Sahni & Bennet.), from Jambi, Indonesia using ITS and MatK gene. International Journal of Environment, Agriculture and Biotechnology (IJEAB), 3:441-446.

Tanaka, T., Koyano, T., Kowithayakorn, T., Fujimoto, H., Okuyama, E., Hayashi, M. and Ishibashi, M. (2001). New multiflorane-type triterpenoid acids from *Sandoricum indicum*. Journal of natural products, 64:1243-1245.

Te-chato, S., Lim, M. and Masahiro, M. (2005). Comparison of cultivar identification methods of longkong, langsat and duku: Lansium spp. Songklanakarin J. Sci. Technol, 27:465-472.

Wijaya, M. D. (2022). Ethnomedicinal, Phytochemicals, and Pharmacological Aspects of Sentul (*Sandoricum koetjape*). Biology, Medicine, & Natural Product Chemistry, 11:65-73.

Wongprasert, T., Phengklai, C. and Boonthavikoon, T. (2011). A synoptic account of the Meliaceae of Thailand. Thai Forest Bulletin (Botany), 39:210-266.

Yadav, R., Pednekar, A., Avalaskar, A., Rathi, M. and Rewachandani, Y. (2015). A comprehensive review on Meliaceae family. World Journal of Pharmaceutical Sciences, 3:1572-1577.

Yulita, K. S. (2011). Genetic variation of Lansium domesticum Corr. accessions from Java, Sumatra and Ceram based on Random Amplified Polymorphic DNA fingerprints. Biodiv, 12:125-130.

(Received: 12 April 2024, Revised: 27 June 2024, Accepted: 30 June 2024)