BIODIVERSITAS
Volume 17, Number 1, April 2016
Pages: 539-543

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d170221

Sequence-Related Amplified Polymorphism (SRAP) analysis for studying genetic characterization of *Bouea macrophylla*

SOMBHAT KAEWPONGUMPAI[1], SUPATTRA POEAIM[1], ONGKARN VANIJAJIVA[2,♥]
[1]Department of Biology, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang (KMITL), Ladkrabang, Bangkok, 10520, Thailand
[2] Faculty of Science and Technology, Phranakhon Rajabhat University, Bangkok, 10220, Thailand. Tel./fax. +66-271-663375, ♥email: vanijajiva@gmail.com

Manuscript received: 17 December 2015. Revision accepted: 29 June 2016.

Abstract. *Kaewpongumpai S, Poeaim S, Vanijajiva O. 2016. Sequence-Related Amplified Polymorphism (SRAP) analysis for studying genetic characterization of* Bouea macrophylla. *Biodiversitas 17: 539-543. Bouea macrophylla* Griff. is well-known as one of native typical fruits in Southeast Asia which needs to be preserved and continuously cultivated because of economical and ecological significances. More recently, sequence-related amplified polymorphism (SRAP) markers have been developed, which are used to amplify coding regions of DNA with primers targeting open reading frames. This technique has proven to be robust and highly variable and is attained through a significantly less technically demanding process. In this research, SRAP method was preliminary applied to assess genetic characterization of *B. macrophylla*. Genomic DNA was extracted from fresh leaf samples. The result clearly showed that at 100 ng template DNA and $MgCl_2$ 5 mM concentration are suitable for further PCR analysis. Thirty SRAP primer combinations were initially screened for analysis and 26 primer combinations were chosen for further analysis. A total of 222 DNA fragments, varying from 90-2500 bp, were amplified. The produced band number for each optimal primer set ranged from 3 to 12 with a percentage of polymorphic bands spanning from 33.33 to 80.00%. Therefore, SRAP analysis is suitable for further analysis method on genetic study of *Bouea* species and related genera.

Keywords: *Bouea macrophylla*, SRAP, genetic characterization

INTRODUCTION

Bouea macrophylla Griff. is a tropical fruit tree indigenous to Southeast Asia. The species belongs to the cashew family (Anacardiaceae) (Chayamarit 2010). In appearance it closely resembles the mango, to which it is related, but its size, foliage and fruit are all smaller. It is commonly known as Marian plum or plum mango, also called ramania or gandaria in Indonesia and kundang, rembunia or setar in Malaysia, mayun in Myanmar, and maprang, mayong or mayongchid in Thailand, respectively (Lim 2012; Rajan et al. 2014). The species is one of the oldest fruit crops, which has been cultivated in Southeast Asia region for more than hundred years. The immature fruit is pale green when the fruit is small and becomes dark green as the fruit develops. The ripe fruit is yellow-orange, mango-like in character, roundish, and juicy with a sour to sweet taste according to the variety, and has a faint turpentine smell. There is one seed in a fruit; the seed is similar to that of the mango but smaller in size (Rifai 1992). The endosperm is white and pinkish purple, and has a bitter and astringent taste. Ripe fruits are consumed fresh, but sometimes they are made of whole or pieces of fruit in sugar syrup. On the other hand, unripe fruits are also consumed by local people as an ingredient of chillies paste as well as traditional salad dish. Many researchers found that an extract of unripe and ripe fruit exhibited various bioactive compound and antioxidant activity (Khoo et al. 2008; Rajan et al. 2014, 2016).

With the introduction of sweet-flesh ripe fruits, *B. macrophylla* has received more attention in recent years. This edible fruit species may have good potential for commercial development if subjected to more research on marketing and postharvest storage. Marian plum is gaining popularity among local consumers in recent years particular in ASEAN countries. The Thai government, for example, is trying to help in exporting this fruit as many exporter firms have started to advertise Marian plum fruit for export. This suggests that *B. macrophylla* has good prospects for wider commercialization. Over the centuries, various *B. macrophylla* cultivars have arisen in Thailand. The plant is normally cultivated in small-scale mixed orchards and is usually grown together with other economic crops and usually sold at local markets when in season (Subhadrabandhu 2001). *B. macrophylla* used to be grown with mixed results from seeds of trees bearing superior quality fruit, but are now propagated by layering, or more commonly, by grafting, including bud, veneer, wedge, whip or grafting onto seedlings of randomly selected rootstocks. One of the reasons for this is the selection of cultivars with high quality fruits. Growers in the central and lower northern regions of Thailand cultivate these high quality Marian plum cultivars in their orchards. More than 50 *B. macrophylla* cultivars have been named in Thailand. However, the difference between its cultivars is practically not studied. There is not much information available on the genetic characterization between cultivated Marian plum cultivars in Thailand.

Knowledge of genetic characterization within crop species is a fundamental resource, which has been employed in breeding programs for the improvement of the crops. Detection of polymorphism among germplasm collections for selected species will provide insight into the genome evolution, origin of cultivated species, and current level of diversity in modern agricultural crops. One of the most promising is the molecular marker technique as it offers great possible to the analysis of plant genetic structure, diversity, and functionality that are required for marker assisted breeding schemes. Nowadays, molecular markers have been incorporated in conventional breeding programs or utilized as a substitute for conventional phenotypic selection on the assumption that efficiency and precision of the genetic improvement could be greatly increased. The available molecular marker techniques include random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), inter primer binding site (iPBS), inter simple sequence repeat (ISSR), and simple sequence repeat (SSR) and sequence-related amplified polymorphism (SRAP) (Agarwal et al. 2008; Kalendar et al. 2010; Zheng et al. 2015). Of these, the SRAP technique is recognized as a simple, efficient, and cost-effective marker system that could be used in multiple molecular biology studies, including genetic-diversity analysis, genomic and cDNA fingerprinting, map construction, gene tagging, and map-based cloning. Compared to other marker systems, this technique is specifically targeted to genome open reading frame (ORF) sequences, which provide more genetic information associated with phenotypes. For successful application of SRAP, the most critical step is the selection of optimal forward and reverse primer pairs that permit an effective polymorphism characterization of various fruit species, such as apple (Si et al. 2010), pear (Zhang et al. 2013), citrus (Hazarika et al. 2014) and guava (Padmakar et al. 2015).

The objectives of this study was to survey the variability of *B. macrophylla* collected from major cultivated area in Thailand and to evaluate the availability of SRAP technique in terms of genetic characterization and significant marker-trait associations, aiming to profile these cultivars properly for further utilize. Currently, no specific SRAP marker is available for Anacardiaceae including this species, and the development of a new marker for this species would be time-consuming and costly. Therefore a more practical approach is to use SRAP marker on *Bouea.* To our knowledge, this is the first report on application of SRAP marker to detect variations among *Bouea* species and related genera.

MATERIALS AND METHODS

Plant materials

Bouea macrophylla was mainly collected from central and lower northern regions of Thailand (Table 1). As for the 30 accessions collected in our survey, 29 samples are under cultivation and one sample (MP18) is uncultivated. The obtained fresh leaves were stored at -20 °C until further processing.

Table 1. The names *Bouea macrophylla* cultivar and origins of samples for SRAP analysis

Cultivar name	Origin	Code
Maprang-Mae Anong	Nakhon Nayok, Thailand	MP01
Maprang-Thong Nopparat	Nakhon Nayok, Thailand	MP02
Maprang-Thong Yai	Nakhon Nayok, Thailand	MP03
Maprang-Maha Chanok	Nakhon Nayok, Thailand	MP04
Maprang-Suwan Nabat	Nakhon Nayok, Thailand	MP05
Maprang-Chao Sua	Nakhon Nayok, Thailand	MP06
Maprang-Lung Chit	Nakhon Nayok, Thailand	MP07
Maprang-Waan Yai	Phetchabun, Thailand	MP08
Maprang-Waan Thong	Phetchabun, Thailand	MP09
Maprang-Patum Thong	Sukothai, Thailand	MP10
Maprang-Rung Arun	Prachinburi, Thailand	MP11
Maprang-Phet Wan Yao	Kamphaengphet, Thailand	MP12
Maprang-Waan Kom	Kamphaengphet, Thailand	MP13
Maprang-Puang	Phetchabun, Thailand	MP14
Maprang-Phet Noppakao	Kamphaengphet, Thailand	MP15
Maprang-Cheong Lan	Kamphaengphet, Thailand	MP16
Maprang-Yai	Phetchabun, Thailand	MP17
Maprang	Ayutaya, Thailand	MP18
Mayongchid-Suan Waan	Nakhon Nayok, Thailand	MY01
Mayongchid-Chit Sanga	Nakhon Nayok, Thailand	MY02
Mayongchid-Tan Kao	Nakhon Nayok, Thailand	MY03
Mayongchid-Bang Khun Non	Nakhon Nayok, Thailand	MY04
Mayongchid-Tan Tawai	Nakhon Nayok, Thailand	MY05
Mayongchid-Tadaan	Nakhon Nayok, Thailand	MY06
Mayongchid-Tan Kao	Lopburi, Thailand	MY07
Mayongchid-Neang Siam	Sukothai, Thailand	MY08
Mayongchid-Mae Ya	Sukothai, Thailand	MY09
Mayongchid-Bang Khun Non	Kamphaengphet, Thailand	MY10
Mayongchid-Phet Kang Dong	Kamphaengphet, Thailand	MY11
Mayongchid-Phet Cheong Lan	Kamphaengphet, Thailand	MY12

Genomic DNA isolation

Total genomic DNA was extracted individually from young leaves of 30 accessions using the CTAB method (Doyle and Doyle, 1987) with minor modification. The leaves (500 mg) were ground in a mortar with a pestle. Extraction buffer [(1% (w/v) CTAB, 50 mM Tris-HCl (pH 8), 0.7 M NaCl, 0.1% -mercaptoethanol)] 500 µL was added and the solution was incubated at 60 ºC for 30 min. The homogenate was mixed with 25: 24: 1 phenol: chloroform: isoamyl alcohol (v/v/v) by gentle inversion. After centrifugation at 13,000 rpm for 15 min, the upper aqueous layer was transferred to a fresh tube. RNA was removed by treating with 2.5 µL of the RNase (10 µg/µl) for 30 min at 37 ºC. The extraction of DNA with phenol/chloroform/isoamyl alcohol was repeated one more time. DNA in the solution was precipitated with 0.6 volume of ice-cold isopropanol and washed with 70% ethanol. Following this, the DNA was extracted using CTAB DNA extraction protocol without RNase. The process was repeated until the DNA pellet was free of color (two to three times) and the final pellet was dissolved in sterile deionized water. DNA quality and quantity were determined on 0.8% agarose gel. The DNA was stored at -20 ºC, for further use as templates for PCR amplification. The quality of DNA was also evaluated by reading the absorbance at 260 and 280 nm.

Table 1. SRAP primers used in this study

Forward primer	Sequence (5'-3')
me1	TGAGTCCAAACCGGATA
me2	TGAGTCCAAACCGGAGC
me3	TGAGTCCAAACCGGAAT
me4	TGAGTCCAAACCGGACC
me5	TGAGTCCAAACCGGAAG
Reverse primer	**Sequence (5'-3')**
em1	GACTGCGTACGAATTAAT
em2	GACTGCGTACGAATTTGC
em3	GACTGCGTACGAATTGAC
em4	GACTGCGTACGAATTTGA
em5	GACTGCGTACGAATTAAC
em6	GACTGCGTACGAATTGCA

SRAP analysis

Primers pairs used in this study were synthesized by Ward Medic Ltd., Part. Thailand (Table 1). The PCR was performed using a Thermohybaid Px2 (Roche Molecular Systems, Inc., USA). The PCR reaction mixtures (25 µL total volumes) consisted of 10x Reaction Buffer, 100 ng template DNA, 0.6 mM dNTP mixture, 5 mM $MgCl_2$, 1 unit of Taq polymerase and 0.6 µM of each primers. The SRAP amplification conditions were 5 min initial denaturation at 94ºC and 5 cycles consisting of 1 min denaturation at 94ºC, 1 min primer annealing at 35ºC, and 2 min extension at 72 ºC. In the following 30 cycles, the annealing temperature was increased to 50°C and a final 8 min extension at 72 ºC.

The SRAP products were all analysed by agarose (1.8% w/v) gel electrophoresis at 150 volts for 30 minutes in 0.04 M TAE (Tris-acetate 0.001 M-EDTA) buffer pH 8. The gels were stained with ethidium bromide (10 mg/ml). The gels were viewed and photographed by Bio-Imaging System (Syngene, Genegenuis). To determine SRAP profiles, the size of each DNA band was inferred by comparison with a 100 bp DNA ladder (Promega), used as a molecular weight marker (M). Polymorphisms at all loci were confirmed by three repeating tests for each primer at different times.

RESULTS AND DISCUSSION

DNA isolation and optimization of SRAP-PCR parameters

The extraction of high quality DNA from *Bouea macrophylla* is challenging because presence of high polyphenolics in the tissues. A high throughput DNA extraction protocol is prerequisite. The presence of polyphenols, which are influential oxidizing agents show in many tropical plant species, can decrease the yield and purity by binding covalently with the extracted DNA making it useless of most research applications (Vanijajiva 2011). The extraction of high quality DNA was optimized by re-extracting the DNA using CTAB DNA isolation protocol and phenol: chloroform: isoamyl alcohol extraction instead of chloroform: isoamyl alcohol extraction. The polyphenolics with the DNA were simply removed and good SRAP electrophoretograms were obtained with all samples. DNA extracted from *B. macrophylla* leaf using an above modified gave a good and sufficient quality DNA for SRAP-PCR reaction. DNA isolated by minor modification method yielded strong and reliable amplification products and the amount of DNA extracted from the accessions ranged from 125 to 245 µg/g fresh weight leaf material. The ratios of A260/A280 varied from 1.84 to 1.98. The quality of DNA was also tested by PCR, which confirmed that the DNAs were suitable for PCR reaction. The parameters for the sequence-related amplified polymorphism protocol from *B. macrophylla* cultivars were also studied. Several parameters had an effect on banding patterns and reproducibility such as concentration of dNTPs, magnesium chloride concentration, concentration of enzyme, concentration of primer and concentration of template DNA (Sun et al. 2011), but the concentration of template DNA and magnesium chloride were most important. The result clearly showed that at 100 ng template DNA and $MgCl_2$ 5 mM concentration are suitable for further PCR analysis.

SRAP analysis

Genetic characterization is one of the key successes to crop breeding programs. Knowledge of the genetic variation between the different accessions supplying this diversity can greatly assist the development of efficient germplasm-management and -utilization strategies. Currently, genetic marker technology designed to detect naturally occurring polymorphisms at the DNA level had become an invaluable and revolutionizing tool for both applied and basic studies of plant. In this study, *Bouea macrophylla* was used for analyzing molecular characterization using a novel molecular marker sequence-related amplified polymorphism (SRAP). The selected primers were based on earlier reports of Li and Quiros (2001) and Vanijajiva and Kundee (2014). There were 30 sets of primer combinations that combined 5 forward primers and 6 reverse primers. Based on preliminary test, 26 sets of combination primers which steadily produced well-defined and scorable amplification products showed polymorphisms in all 30 *B. macrophylla* cultivars. Figure 1 was the illustration amplification electrophoretograms of MP18 accession of *B. macrophylla*.

Total and polymorphic band number and polymorphism ratio of *Bouea macrophylla* accessions were processed in Table 2.The main criteria by which the primer selection was made are: clarity, reproducibility of amplified bands and high rates of polymorphism. The 26 primer combinations generated 222 electrophoretic profiles, of which 150 bands were polymorphic (57.27%). A high degree of polymorphism was revealed by these combinations that ranged from 33.33 to 80.00% across all the genotypes studied. The size of amplified bands ranged from 90 to 2,500 bp. The number of fragments amplified by each primer ranged from 3 to 12 with the average of 8.53 per primer combination. By scoring the bands from forward or reverse primer directed primer combinations, the results showed that the amplification ability of either

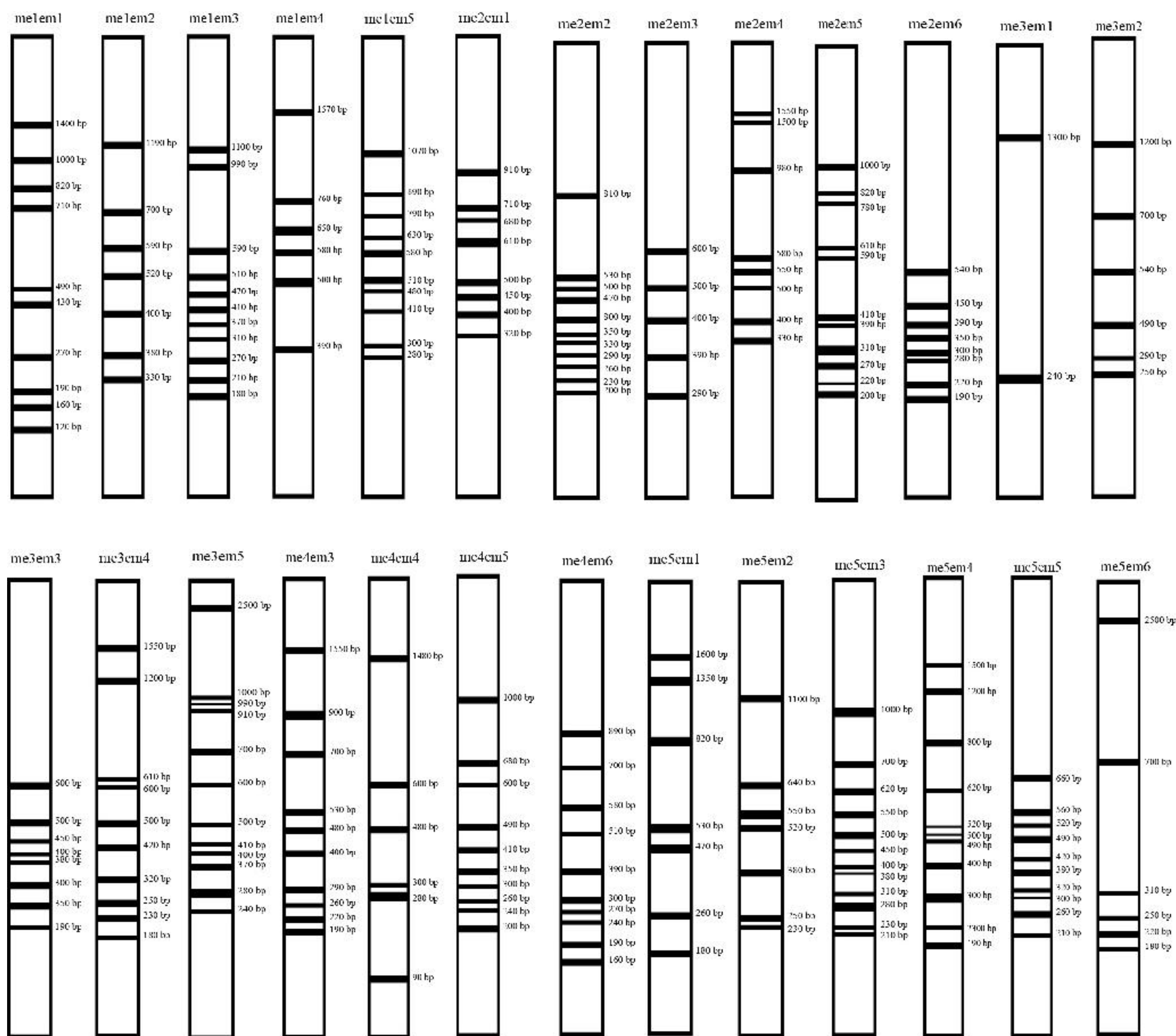

Figure 1. The representative SRAP profiles in this study. The electrophoretograms are employed as representative of clear, distinguished, stable profiles from 26 combination primers of MP18 wild accession

the forward or reverse primers varied significantly with each other, revealing a genomic bias of *B. macrophylla* cultivars on different forward- and reverse-primer nucleotides. In fact, primer preference for successful SRAP-PCR assays has been widely recognized in many plant species (Agarwal et al. 2008).

This preliminary result indicated that the SRAP technique could be used as an alternative molecular tool on *B. macrophylla*. Recently, ISSR and SSR has been applied in the molecular biology studies of *Bouea* species (Damodaran et al. 2013; Ghazali et al. 2015). However, SSR and ISSR techniques provided limited functional gene information associated with the traits of interest. In other plant species, such as buffalograss cultivars, SRAP technology prove useful for varietal identification than SSR and ISSR markers (Budak et al. 2004) as well as *Dianthus* accessions, the information given by SRAP markers was more concordant to the morphological variability and to the evolutionary history of the morphotypes than that of ISSR markers (Fu et al. 2008). These contrasting levels of SRAP, SSR and ISSR correlation with morphological traits may be related to the fact that, unlike SSR and ISSR which are targeted to microsatellite regions, SRAP markers preferentially amplify ORFs (open reading frames). Exons are usually GC rich and, thus, the 'CCGG' sequence in the core of the forward SRAP primers is designed to target such coding regions (Li and Quiros 2001; Shao et al. 2010). Thus, SRAP technique may be helpful in deciphering the genomic basis of complex traits that are related to the economic value of *B. macrophylla* and are likely to better reflect genetically determined morphological variation.

Table 2. Total and polymorphic band number and polymorphism ratio of *Bouea macrophylla* accessions

Primer combinations	Total bands	Poly-morphic bands	% Poly-morphism	Size (bp)
me1em1	10	7	70.00	120-1400
me1em2	7	6	85.71	330-1190
me1em3	11	8	72.72	180-1100
me1em4	6	3	50.00	390-1570
me1em5	10	7	70.00	280-1070
me2em1	8	6	75.00	320-910
me2em2	11	6	54.55	200-810
me2em3	5	4	80.00	290-600
me2em4	8	3	37.50	330-1550
me2em5	11	8	72.73	200-1000
me2em6	8	6	75.00	190-540
me3em1	3	1	33.33	240-1300
me3em2	6	1	16.67	250-1200
me3em3	8	3	37.50	190-600
me3em4	10	7	70.00	180-1550
me3em5	11	8	72.72	240-2500
me4em3	10	4	40.00	190-1550
me4em4	6	4	66.67	90-1480
me4em5	10	8	80.00	200-1000
me4em6	10	2	20.00	160-890
me5em1	7	5	71.43	180-1600
me5em2	7	3	42.86	230-1100
me5em3	12	8	66.67	210-1000
me5em4	11	6	54.55	190-1500
me5em5	10	4	40.00	210-660
me5em6	6	2	33.33	180-2500
Total	222	130		90-2500
Mean	8.53	5	57.27	

In conclusion, the present study is, to the best of our knowledge, the first report of genetic investigation of *Bouea macrophylla*, using SRAP markers. It is concluded that SRAP is a useful DNA fingerprinting tool for evaluation of genetic diversity of species, cultivars and breeding lines, especially for species with underdeveloped marker systems. It is a fast, low-cost and efficient molecular method applicable to plant breeding.

ACKNOWLEDGEMENTS

The authors are grateful to Vichit Kaisornsawad and Amnuay Hongthong for supplying the plant materials. This work was supported by Mongkut's Institute of Technology Ladkrabang, Thailand and Phranakhon Rajabhat University, Thailand.

REFERENCES

Agarwal M, Shrivastava N, Padh H. 2008. Advances in molecular marker techniques and their applications in plant sciences. Pl Cell Rep 27: 617-631.

Budak H, Shearman RC, Parmaksiz I, Dweikat I. 2004. Comparative analysis of seeded and vegetative biotype buffalograsses based on phylogenetic relationship using ISSRs, SSRs, RAPDs, and SRAPs. Theor Appl Genet 109: 280-288.

Chayamarit K. 2010. Anacardiaceae. In: Santisuk T, Larsen K (eds) Flora of Thailand 10(3). Prachachon, Bangkok.

Damodaran T, Ahmad I, Nagarajan B. 2013. *Bouea oppositifolia*-A fast disappearing native mango genetic resource from Andamans: Morphological and molecular evidences. Indian J Hort 70: 161-164.

Doyle JJ, Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19: 11-15.

Fu X, Ning G, Gao L, Bao M. 2008. Genetic diversity of *Dianthus* accessions as assessed using two molecular marker systems (SRAPs and ISSRs) and morphological traits. Scientia Horticulturae 117: 263-270.

Ghazali MN, Yunus MF, Mohammad AL. 2015. Assessment of genetic relationships within *Bouea* (Anacardiaceae) accessions in Peninsular Malaysia using inter simple sequence repeats (ISSR) markers. African J Biotechnol 14: 76-85.

Hazarika TK, Hazarika BN, Shukla AC. 2014. Genetic variability and phylogenetic relationships studies of genus *Citrus* L. with the application of molecular markers. Genetic Res Crop Evol 61 (8): 1441-1454.

Kalendar R, Antonius K, Smykal P, Schulman AH. 2010. iPBS: a universal method for DNA fingerprinting and retrotransposon isolation. Theor Appl Genet 121: 1419-1430.

Khoo HE, Ismail A, Mohd-Esa N, Idris S. 2008. Carotenoid content of underutilized tropical fruits. Pl Food Human Nutr 63: 170-175.

Li G, Quiros CF. 2001. Sequence related amplifi ed 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.

Lim TK. 2012. Edible medicinal and non-medicinal plants (Vol. 1, pp. 656-687). Springer.

Padmakar B, Kanupriya C, Latha PM, Prashant KS, Dinesh MR, Sailaja D, Aswath C. 2015. Development of SRAP and SSR marker-based genetic linkage maps of guava (*Psidium guajava* L.). Scientia Horticulturae 192: 158-165.

Rajan NS, Bhat R, Karim AA. 2014. Preliminary studies on the evaluation of nutritional composition of unripe and ripe Kundang fruits (*Bouea macrophylla* Griffith). Intl Food Res J 21: 949-954.

Rajan NS, Bhat R. 2016. Antioxidant compounds and antioxidant activities in unripe and ripe kundang fruits (*Bouea macrophylla* Griffith). Fruits 71: 41-47.

Rifai MA. 1992. *Bouea macrophylla* Griffith. In: Coronel RE, Verheij EWM (eds.) Plant resources of South-East Asia. No. 2: Edible fruits and nuts. Prosea Foundation, Bogor.

Shao QS, Guo QS, Deng YM,, Guo HP. 2010. A comparative analysis of genetic diversity in medicinal *Chrysanthemum morifolium* based on morphology, ISSR and SRAP markers. Biochem Syst Ecol 38: 1160-1169.

Si P, Dai H Y. 2010. Establishment of SRAP-PCR reaction system in apple. J Fruit Sci 27(2): 168-173.

Subhadrabandhu S. 2001. Under utilized tropical fruits of Thailand. FAO Rap publication 2001/26, Rome.

Sun F, Huo JW, Qin D. 2011. Establishment and Optimization of SRAP amplification system in *Lonicera caerulea* L. J Northeast Agric Univ 18: 26-31.

Vanijajiva O, Kundee N. 2014. Genetic uniformity of exotic medicinal plant *Gynura divaricata* in Thailand. Phranakhon Rajabhat Res J 9: 77-86.

Vanijajiva O. 2011. Genetic variability among durian (*Durio zibethinus* Murr.) cultivars in the Nonthaburi province, Thailand detected by RAPD analysis. J Agric Technol 7: 1107-1116.

Zhang RP, Wu J, Li XG, Khan MA, Chen H, Korban SS, Zhang SL. 2013. An AFLP, SRAP, and SSR genetic linkage map and identification of QTLs for fruit traits in pear (*Pyrus* L.). Pl Mol Biol Rep 31: 678-687.

Zheng H, Duan H, Hu D, Wei R., Li Y. 2015. Sequence-related amplified polymorphism primer screening on Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook). J For Res 26: 101-106.