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Genetic Diversity of Durian (*Durio Zibethinus* Murray) Based on SRAP Marker

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Durian (*Durio zibethinus* Murray) is described as "the king of tropical fruit" which is one of the most important commercial fruit crops. In Thailand, there are many different cultivars of durian. Only a few of these are in commercial cultivation that large potential for supply both local and export markets. This research aims to observe the genetic variability and relationship among durian cultivars using Sequence-Related Amplified Polymorphism (SRAP) markers. DNA extraction from leaves of 29 durian cultivars which were collected from Chanthaburi Horticultural Research Center, Chanthaburi Province, Thailand. The result of this study obtained a total of 234 DNA fragments of which 182 fragments (77.78%) were polymorphic from 13 primer combinations of SRAP technique. The result were analyzed the genetic relationship with UPGMA by NTSYS program. There is similarity coefficient ranged from 0.62-0.93. Based on a cut-off point of 0.77 in Dice's similarity coefficient, the 29 durian cultivars were divided into four groups and three individaul. In this study, SRAP showed itself to be the most efficient. Therefore, SRAP marker can be used to examine the genetic diversity of another durian cultivars in future.

Keywords: genetic diversity, durian, *Durio zibethinus*, SRAP marker

Introduction

Durian is one of the most popular and famous local fruits in Southeast Asia, has been cultivated for centuries (Subhadrabandhu and Ketsa, 2001). The fruit is generally referred to as the king of fruits since its extremely delicious taste and nutritional value. Among the thirty recognised *Durio* species, at least four of which found in Thailand: *D. malaccensis* Planch. Ex. Mast., *D. griffithii*

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(Mast.) Bakh., *D. pinangianus* Ridley and *D. zibethinus* Murray. *D. zibethinus* or durian is the only species has been popular for both domestic and international consumption, widely grown commercially and also has an extremely important economic role (Hiranpradit, 2008). In the beginning of planting, usually the growers propagate durian by seeds, resulting in a number of different cultivars. Therefore, there are probably more than 200 cultivars are reported in Thailand. Individual cultivars have a different flavor and odor profile. Most of them have a common name such as Kop Suwan, Thong Yip, Chat Si Thong and Krapuk Thongdee etc. However, there are no economic importance. There are only four cultivars: Monthong, Chanee, KanYao and Kradumthong which are popular and economic importance (Department of Agriculture, 2010).

Although, there are many characteristics, the botanical description are the most similar which unable to identified or separated durian cultivars. Therefore, it relies on molecular markers used in the classification and genetic diversity. Despite its great power, the molecular study of plant genetic diversity are necessary. Molecular markers such as Amplified Fragment Length Polymorphism: AFLP (Sukhotu *et al*., 2009), Simple Sequence Repeat: SSR (Chareonsap *et al.*, 2009), Random Amplification of Polymorphic DNA: RAPD (Vanijajiva, 2011) and inter-simple sequence repeats: ISSR (Vanijajiva, 2012) have been reported. However, Sequence-Related Amplified Polymorphism (SRAP) marker, amplify open reading frames (ORFs) (Li and Quiros, 2001) based on two special primer have not been reported. Therefore, the purpose of this research to determine genetic diversity of durian cultivars using SRAP marker.

Materials and methods

Plant materials and DNA extraction

This study was carried out using twenty-nine durian cultivars leaves that collected from Chanthaburi Horticultural Research Center, Chanthaburi province showed as Table 1. DNA extraction are modify the CTAB protocol followed Doyle and Doyle, (1990). The leaves (1 to 2 g) were ground and mixed with 700 µL of 2X CTAB solotion [2% (w/v) CTAB, 1M Tris-HCl (pH 8), 0.5M EDTA, 1% PVP and 5M NaCl]. Transfer homogeneous solution into a 1.5 ml microcentrifuge tube and add 2 µl of β-mercaptoethanol. Mix gently and incubate at 65 ℃ in a water bath for 4 hour. The homogenate was mixed in equal volume of chloroform: isoamyl alcohol (24:1, v/v) by gentle inversion. After centrifugation at 14,000 rpm at 4 ℃ for 15 min, the upper

aqueous layer was transferred to a fresh microcentrifuge tube. RNA was removed by treating with 2 µl of the RNase (20 mg/ml) for 1 hour at 37 ℃. After that, add 50 µl of 10% CTAB in 0.7M NaCl. The extraction of DNA with chloroform: isoamyl alcohol was repeated one more time. The DNA was precipitated with ice-cold isopropanol. The pellet is washed with ice cold 70% ethanol followed by absolute ethanol. The final step, the pellet was dissolved in TE buffer. The DNA extract quantity and quality were measured using optical density (OD) at 260 nm and 280 nm by spectrophotometer and 1% agarose gel electrophoresis. The DNA was stored at -20 ℃ until further use.

SRAP analysis

The SRAP analysis was followed according to Li and Quiros (2001). The SRAP reaction was carried out in a final volume of 20 µL consists of 1X standard *Taq* reaction buffer (New England BioLabs, USA), 2.5 mM $MgCl_2$ (Vivantis, Malaysia), 1U *Taq* DNA polymerase (New England BioLabs, USA), 200 µM of dNTPs (Vivantis, Malaysia), 1.0 µM of each primer and 100 ng genomic DNA. The PCR amplification program was conducted following the protocol of Abedian *et al.,* (2012): 3 min of initial denaturing at 94 ℃, five cycles of three steps: 1 min of denaturing at 94 ℃, 1 min of annealing at 35 ℃ and 1 min of elongation at 72 ℃, 35 cycles of 94 ℃ for 1 min, 50 ℃ for 1 min, and 72 ℃ for 1 min; followed by a final extension 10 min at 72 ℃. The SRAP products were separated by electrophoresis on 1.5% (w/v) agarose gel in 1X TBE buffer along with the VC 100 bp plus DNA Ladder marker (Vivantis, Malaysia). The gel was stained with ethidium bromide solution and examined under UV light transilluminator.

Data scoring and SRAP analysis

Clear and well resolved bands of the samples were compared with each other and DNA fragments were scored as present (1) or absent (0) from each primer. Dice's similarity coefficients among the 29 genotypes were calculated. The dendrogram was constructed by genetic similarity matrices using the unweighted pair group mean algorithm (UPGMA) method by the NTSYS-pc software package version 2.01e (Rohlf, 2000).

Table 1. Code and cultivar name of twenty-nine durian cultivars used in this study

Code	Cultivar name	Code	Cultivar name	Code	Cultivar name
DZ05	Jao Noge	DZ 24	Chai Mafai	DZ 37	Kop Chainam
DZ06	Nom Sawan	DZ 25	Chat Si Thong	DZ 38	E-ngon
DZ 07	Krapuk Thongdee	DZ 26	Chat Si Thong	DZ 39	Nok Yip
DZ 08	Saochom Fakthong	DZ 27	Maled Kotchasarn	DZ 40	Tonyai
DZ 12	Lueang Thong	DZ 28	Thong Yoi Doem	DZ 41	E-lip
DZ 13	Kop Suwan	DZ 29	Kanyao Si-nak	DZ 42	Yindi
DZ 15	Metnai Yaiprang	DZ 30	Pin Thong	DZ 43	Saochomhet
DZ 18	Kop Watklual	DZ 33	Kop Nasan	DZ 44	Eai-Mai
DZ 21	Kop Langwihan	DZ 34	Kop Thongpheng	DZ 45	Thunthawai
DZ 23	Thong Yip	DZ 35	Kop Chaokhun		

Results and Discussions

SRAP amplification

In preliminary, thirty sets of primer combinations that were combined by 5 forward primers and 6 reverse primers were screened, out of which only 13 primer combinations showed reproducible fragments with easily recordable bands and gave polymorphisms (Thinhuatoey *et al*., 2016). In this study, determine the genetic diversity among twenty nine durian cultivars with the thirteen SRAP primer combinations that able to produce intense bands in all samples. The SRAP profiles of 29 samples from ME1/EM1 and ME4/EM5 primer combinations shown in figue 1A and 1B, respectively. From thirteen primer combination, the total of 234 DNA fragments were observed. The number of SRAP bands per primer combinations ranged from 15 (ME5/EM2) to 21 (ME4/EM5) with an average 18. ME5/EM2 primers gave the highest number of polymorphic fragments (86.87 %). However, in the total 182 polymorphic bands were scored with an average 14 and percentage of polymorphism (% polymorphism) is 77.78% (Table 2).

SRAP relationship between cultivars

The dendrogram was constructed from the distance matrix based on Dice's similarity coefficients. The dendrogram by UPGMA analysis, similarity coefficient ranged from 0.62 to 0.93. Based on a cut-off point of 0.77 in Dice's similarity coefficient scale, the twenty nine durian cultivars were divided into four groups and three individaul. The first group contained only two cultivars (DZ05 and DZ06). The major group II divided into three sub-groups. The first sub-group consist of four cultivars: DZ07, DZ25, DZ26 and DZ44.

Inside the second sub-group were six cultivars: DZ08, DZ21, DZ29, DZ45, DZ15 and DZ23, with close relationship to third sub-group consist of Kob cultivars (DZ13, DZ18, DZ33, DZ35 and DZ37). The group III and IV contained each two cultivars, which have close relationship lower other groups. The section of three individual consisted of DZ39, DZ40 and DZ43 (Figue 2).

Classification can be residents different of morphology to a certain extent but durian have several cultivars. Durian cultivars have been divided into six groups based on fruit morphology which consists of leaf shape, leaf apex, leaf base, fruit and thorn, (Hiranpradit *et al*., 2003). There are Kob group, Luang group, Kan Yao group, Kam Pan group, Thong Yoi group and Miscellaneous group. The durian samples used in this study were classified, rather in the same way as morphology. Kob group, Thong Yoi group and Miscellaneous group considering the leaf shape and leaf base that each group has several forms, which were classified close together group. In group II cultivars are in the majority of Kob group, Thong Yoi group and Miscellaneous group. Generally, the Miscellaneous group have many different morphological variation, may be similar with Kob and Thong Yoi group. However, Kop Thongpheng (DZ34) may be differences, it was separated from the Kob group.

Vanijajiva (2011 and 2012) using RAPD and ISSR markers in the same caltivars from Nonthaburi province showed ISSR marker are more interspecific diversity than RAPD. Including, Sukhotu *et al*., (2009) using AFLP marker to identification and classification of durian from Chanthaburi Horticultural Research Center, that showed high polymorphism about 83.64% and two major clusters were detected. In this study, we used samples in same cultivars for determination genetic diversity which results were agreeable with the AFLP marker such as Chat Si Thong has a close relationship with Krapuk Thongdee. Including, Maled Kotchasarn have a closer relationship with Kop Chaokhun.

Table 2. Primer codes combination, the number of total DNA bands, the number of polymorphic bands and the percentage of polymorphism detected by the SRAP marker in 29 durain cultivars

Primer codes combination	No. of total DNA bands	No. of polymorphic bands	% of polymorphism
ME1/EM1	17	12	70.59
ME1/EM4	20	17	85.00
ME2/EM1	17	14	82.35
ME3/EM3	18	11	61.11
ME3/EM4	16	12	75.00
ME3/EM5	19	15	78.95
ME3/EM6	20	17	85.00
ME4/EM2	16	12	75.00
ME4/EM5	21	18	85.71
ME5/EM1	17	10	58.82
ME5/EM2	15	13	86.87
ME5/EM3	19	15	78.95
ME5/EM5	18	15	83.33
Total	**234**	**182**	**-**
Average	**18**	**14**	**77.78**

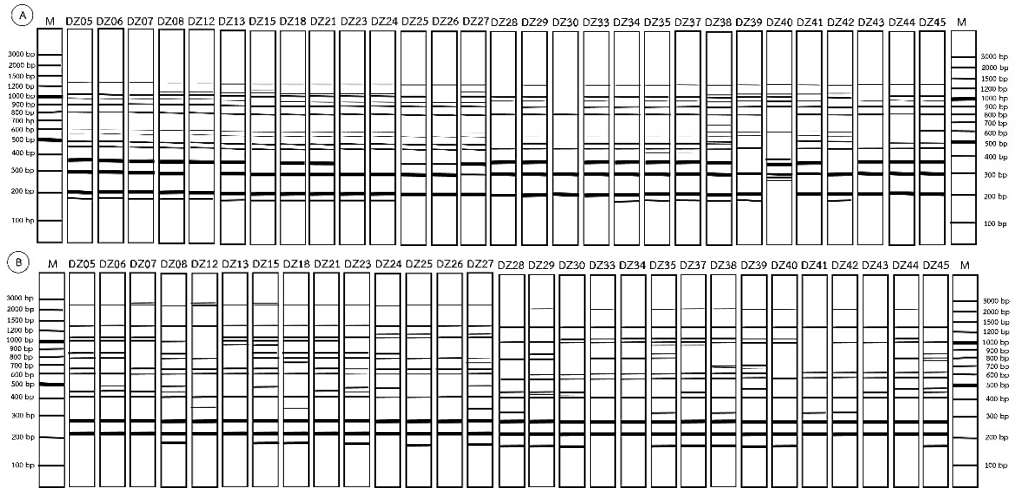

Figure 1. Examples of the SRAP profiles from 29 durian cultivars revealed by SRAP primer combinations (A) ME1/EM1 and (B) ME4/EM5 (left to right: Lane M: VC 100 bp plus DNA Ladder, Lane DZ codes: genomic DNA isolated from different durian cultivars. The numbers correspond the code of durian is given in Table 1)

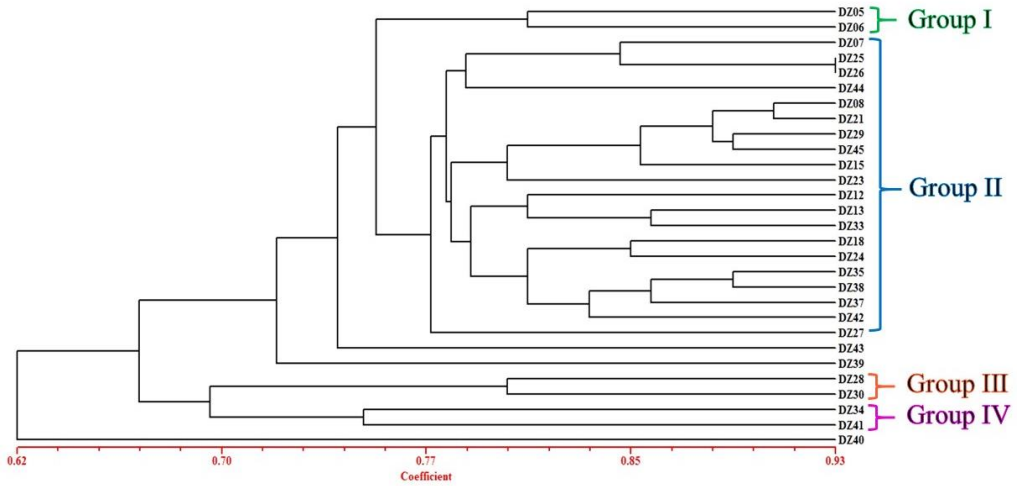

Figure 2. Dendrogram of 29 durian cultivars constructed from SRAP data using UPGMA method based on Dice's similarity coefficient

Conclusion

For SRAP marker, determination of genetic diversity among twenty nine durian cultivars with the thirteen SRAP primer combinations that percentage of polymorphism is 77.78%. ME5/EM2 primer combinations gave the highest number of polymorphic (86.87 %). The similarity coefficient ranged from 0.62 to 0.93, revealed medium level of genetic diversity. However, based on a cut-off point of 0.77 in similarity coefficient were divided into four groups and three individaul. The cultivars in Kob group are classified and clearly isolated from other groups. The results from this study indicate that SRAP marker is efficiency and stable similar to AFLP marker, simple as well as faster. Therefore, this study provides evidence that SRAP marker could be used for future determination in large durian population.

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