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ASSESSMENT OF GENETIC VARIABILITY OF OPEN-POLLINATED OIL PALM IN SOUTHERN THAILAND USING SSR MARKERS

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ABSTRACT

Genetic variability of plant materials is very crucial when they are used for breeding purpose. In this present study, genetic variation of 100 open pollinated seeds of *tenera* hybrid (*dura* x *pisifera*) from five provinces in southern Thailand including Phang-nga, Krabi, Surat Thani, Trang and Chumphon were assessed using SSR markers. Seven SSR primers produced 20 alleles with the average of 2.86 alleles per locus. AMOVA analysis based on sampling locations indicated that 99% of genetic variation was observed within populations rather than among populations. Therefore, oil palm populations from different provinces were not significantly diverged. The genetic relationships among oil palm genotypes were further analyzed using cluster analysis and PCoA based on Jaccard's similarity coefficient. One hundred oil palms were clustered into two groups, regardless of sampling locations. These analyses are useful information for establishing material for breeding program and for crossing scheme. High genetic variability to be included in breeding population can be achieved by selecting oil palms from two different clusters identified.

Keywords; Oil palm, Genetic variation, SSR markers, Microsatellites

INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) is an important oil crop grown preliminary for supplying both food (i.e. margarine, cooking oil) and non-food (i.e. cosmetics, biodiesel) industries. Oil palm is currently cultivated in more than 40 countries worldwide, contributing approximately to 20 million hectares of harvesting areas (Pirker *et al*., 2016; Vijay *et al*., 2016). Oil palm is originated from the tropical rainforest of West Africa and was introduced to Southeast Asia in 1848. Starting from four oil palm seedlings planted at the botanical garden in Java, Indonesia, the offspring from those plants were distributed to Deli, Sumatra Island, Indonesia between 1853 and 1856 and to Rantau Panjang, Kuala Selanor, Malaysia (Barcelos *et al*., 2015; Corley *et al*., 2003; Basiron, 2002; Corley and Tinker, 2003). Oil palm was introduced to Thailand from Malaysia in 1937. The first oil palm plantation was in Songkhla province and the commercial plantations using seedlings from Malaysia were spread across the region.

Three oil palm varieties (*dura*, *pisifera*, and *tenera*) are found with the differences in the presence and thickness of fruit kernel shell. In the early years of oil palm cultivation, the common variety widely grown was *dura*, a variety with thick shell, thin layer of mesocarp and low oil content. Nowadays, however, the most widely grown throughout Southeast Asia is *tenera* variety which is produced by crossing between *dura* and *pisifera*. *Tenera* is characterized by thick layer of mesocarp and a thin shell (0.5–3 mm) enclosed by a dark fiber ring. *Pisifera* bears fruit without shell, is usually female sterile, and frequently produces bunches that rot before maturity. Among three varieties, *Tenera* has the highest oil yield (Corley and Tinker, 2003).

Currently, due to rapid increase of the world population and consequently the higher demand of oil palm, areas planted with oil palm are expanding and invading the land with suboptimal growing conditions such as low temperature, long spell of drought, acidic soil and high salinity soil. Therefore, new cultivars suitable for each growing environment have to be developed and bred locally. To achieve successful breeding and cultivar improvement, the assessment of genetic variation of plant materials is needed.

The genetic diversity can be evaluated using morphological and molecular markers. However, diversity based on morphological criteria might not be adequately informative as the number of morphological markers are limited and it is difficult to avoid the influence of environmental factors or the developmental stage of the plant (Govindaraj *et al*., 2015). In contrast, molecular markers such as restriction fragment length polymorphism (RFLP), cleaved amplified polymerphic sequences (CAPS), random amplified polymorphism DNA (RAPD), amplified fragment length (AFLP), and simple sequence repeats (SSR) are abundant and not affected by environmental conditions (Abdalla, 2009; Ashraf and Foolad, 2013; Arif *et al*., 2010; Kumar *et al*., 2009; Hazarika *et al*., 2014; Gimenes *et al.*, 2002; Sharma *et al*., 2011). Among those molecular markers, SSR

has number of advantages over others including high level of allelic variation, being distributed throughout the genome and co-dominant (Miah *et al*., 2013). SSR has been successfully applied to evaluate genetic diversity in multiple crops such as rice (Ravi *et al*., 2003), bread wheat (Warburton *et al*., 2006), peach and sweet cherry (Barac *et al*., 2014; Dirlewanger *et al*., 2002), maize (Yan *et al*., 2009; Matsuoka *et al*., 2002), coconut (Perera *et al*., 2003; Meerow *et al*., 2003) and oil palm (Ting *et al*., 2010; Ting *et al*., 2014; Billotte *et al*., 2005; Singh *et al*., 2008). This present study, SSR markers were used to determine genetic diversity of open-pollinated oil palm collected from southern Thailand to assess the potential of using them as germplasm for breeding purpose.

MATERIALS AND METHODS

Oil palm materials: Open-pollinated oil palm fruits from bunches of *tenera* trees were randomly collected from multiple oil palm fields and loading ramps in Phang-nga (P), Krabi (K), Surat Thani (S), Trang (T) and Chumphon (C) Provinces in southern Thailand. From each loading ramp or oil palm field, 15 open-pollinated fruits were collected. Oil palm pericarps were removed by depericarper. The seeds were then kept at 40 ºC for 60-80 days in temperature-controlled room for dormancy breaking. Oil palm sprouting seeds were sown in nursery tray under shad net for 3 months. Subsequently, three-month old seedlings were transferred to plastic bags and placed under full sunlight with daily irrigation for 6 months. Seedlings with abnormal characteristics were discarded. Twenty oil palm seedlings from each population (province) were tagged randomly as P1 to P20, K1 to K20, S1 to S20, T1 to T20 and C1 to C20, resulting in 100 oil palm seedlings in total.

Genomic DNA extraction: CTAB method was applied to extract DNA from each of the tagged seedlings using approximately 300 mg of young leaves. Leaf tissue was ground with liquid nitrogen using mortar and pestle and transferred to microcentrifuge tube. A total of 700 µl of CTAB extraction buffer with 2% β-mercaptoethanol was added to the tube and the mixture was then incubated at 60 ºC for an hour. Subsequently, 800 µl of chloroform was added to the tube prior to centrifugation at 13,000 rpm for 10 mins. The supernatant portion was transferred to a new 1.5 ml microcentrifuge tube for DNA precipitation using 600 µl of cool isopropyl alcohol. DNA pellet was dissolved with 50 µl of TE buffer. The purified genomic DNA was quantified on Nanodrop and was subsequently adjusted the concentration to 50 ng/µl for polymerase chain reaction.

Amplification of SSR markers: Seven SSR markers developed by Abdullah *et al*. (2011) were used in the present study. The sequences and length of primers as well as the repeat units are given in table 1. Optimal annealing temperature for each primer was determined using gradient PCR. SSR markers were amplified in a total of 12.5 µl reaction mixture containing 1.25 µl of 10x taq buffer, 0.25 µl of dNTP mixed (2 mM each), 0.25 µl of 10 mM forward primer, 0.25 µl of 10 mM reverse primer, 1 µl of 25 mM $MgCl_2$, 0.06 µl of taq polymerase, 8.44 µl of Diethylpyrocarbonate (DEPC) water and 1 µl of DNA template. The PCR amplification was performed as followed: pre-denaturation at 95 ºC for 30 sec, 30 cycles of denaturation at 95 ºC for 30 sec, annealing at 52 or 54 ºC (depends on primer) for 30 sec, extension at 68 ºC for 30 sec, final extension at 68 ºC for 5 mins. The size of PCR products were analyzed using the Microchip Electrophoresis System (MCE-202 MultiNA; Shimadzu, Kyoto, Japan).

Data analysis: The SSR bands were scored and subjected to statistical analysis. Polymorphism information content (PIC) for each locus for SSR markers were calculated as described by Botstein *et al*. (1980) and Anderson *et al*. (1993). Analysis of molecular variance (AMOVA) was performed using GENALEX v. 6.5 (Peakall and Smouse, 2012) to explain genetic variability among and within the oil palm populations based on sampling provinces. Jaccard's similarity coefficients were computed to investigate genetic relationship among oil palm genotypes. An unweighted pair-group method with arithmetic means (UPGMA)-based dendrograms and a plot of the first two principal coordinates were constructed based on Jaccard's similarity coefficient using NTSYS-pc v.2.1 (Rohlf, 2000).

Table 1: Details of SSR primers used

Primers	Sequences	TA (ºC)	Expected size (bp)	Repeat units
CNH00887	F: TTATTGATTGATGCAAGATACAC R: TTGATAAAATACAAGAGATAGCA	52	165	$(AT)_9$
CNH01617	F: TCTTTAATTTGTCGAGGATAATG R: ATGCAAGGTTTTGTTGAAACT	52	130	$(CT)_{20}$
CNI01937	F: AACTGCAAATGAGACACAGAG R: TCCACCAGAGGAGGGTTAGT	52	170	$(AG)_9$

EAP03160	F: AACGTGAGAGCCATAGAGATAG R: TAATAGAAACTAGACCCGACCA	52	175	$(TATG)_6$
MF233033	F: GAGGAGGAGGGGAGAAGAGT R: AAATACCATTCAGAGAAAGCAC	52	200	$(TC)_{11}$
MF233056	F: CCGAATAGAAGAGGAAAGAATA R: AGGTTTGGTGGAGAAGTGTT	52	232	$(CT)_{15}$
MF2331019	F: TGGGTAAATTGGTAATTCTCCT R: CCTTTTTCTTCCTCTTTTCCA	54	195	$(TC)_8$

TA, temperature of amplification. Seven primers used were reported by Abdullah *et al.* (2011)

RESULTS AND DISCUSSION

SSR polymorphisms and genetic variation: SSR markers have become commonly used in the study of genetic relationship and variation in oil palm populations (Abdullah *et al*., 2011; Taeprayoon *et al*., 2016, Ting *et al*., 2010). This present study, all seven SSR primers used showed reproducible amplification and variability among 100 genotypes sampled. A total of 20 alleles were observed from 7 SSR primers. Each primer amplified 2 to 4 alleles with an average of 2.86 alleles per locus (Table 2). Similar number of alleles per locus using the same set of SSR primers in parental palm (*dura* and *pisifera*) and their progenies was reported by (Abdullah *et al*., 2011). The higher number of alleles per locus was reported by Taeprayoon *et al*., (2015) who studied genetic variation of breeding populations from three major oil palm companies in Thailand using 20 SSR markers and observed 3-10 alleles per locus with an average of 5.45 indicating high genetic variation within those populations. Polymorphism information content (PIC) value is frequently used as a measurement of informativeness and polymorphism for a marker locus (Botstein *et al*., 1980). In this present study, CNH00887, CNH01617, EAP03160 and MF23-3056 appeared to be highly informative (PIC>0.5) and suitable for marker-assisted breeding program, whereas CNI01937, MF233033 and MF2331019 were moderately informative (0.5>PIC>0.25) according to Botstein *et al*. (1980) criteria (Table 2). Population-specific alleles were not observed. Each population showed the same number of alleles at all loci studied except for EAP03160. Only three alleles of EAP03160 locus were present in Chumphon population, while other populations contained four alleles. This indicated the close genetic relationship among population studied. Analysis of molecular variance (AMOVA) of open-pollinated oil palm from five provinces revealed that 99% of the molecular variance in the 100 oil palm genotypes exists within populations and only 1% among populations (Table 3). This, therefore, confirmed that oil palm genetic variation was not discrete across different sampling locations (provinces). It is mostly because the oil palms planted in the provinces studied and the rest plantation areas are *tenera* hybrid supplied from the same few oil palm breeding companies in Thailand. Hence, the level of genetic variation among populations of different locations was very low. High genetic divergence was observed among oil palm genotypes. Therefore, the genetic variation and relatedness of 100 oil palm genotypes were further analyzed as the information for establishing genetic materials for breeding program.

Table 2: Number of alleles, number of alleles in each population and PIC of 7 SSR markers used in this study

Primers	Number of alleles	Number of alleles in each population					PIC
		P	K	S	T	C	
CNH00887	3	3	3	3	3	3	0.61
CNH01617	3	3	3	3	3	3	0.59
CNI01937	2	2	2	2	2	2	0.37
EAP03160	4	4	4	4	4	3	0.68
MF233033	2	2	2	2	2	2	0.47
MF233056	4	4	4	4	4	4	0.69
MF2331019	2	2	2	2	2	2	0.34
Total	20	20	20	20	20	19	
Mean	2.86	2.86	2.86	2.86	2.86	2.71	0.54

PIC, polymorphism information content; P, Phang-nga population; K, Krabi population; S, Surat Thani population; T, Trang population; C, Chumphon population

Table 3: Analysis of molecular variance for 100 oil palm genotypes collected from five provinces

Source of variation	df	Sum of squares	Mean square	Estimated variance	% of variation
Among provinces	4	11.100	2.775	0.021	1
Within provinces	195	373.600	1.916	1.916	99
Total	199	384.700		1.937	100

Genetic relatedness among oil palm genotypes based on SSR markers: The seven SSR marker scores of 100 oil palm genotypes were used to determine the genetic distances using Jaccard's similarity coefficient and UPGMA cluster analysis. The average Jaccard's similarity coefficient was 0.46 indicating considerable genetic variation in the oil palm plants collected. A dendrogram separated the oil palm genotypes into 2 groups with each group consisting of oil palm from mixed sampling locations. Group 1 comprised 52 oil palm genotypes from all locations (10 genotypes from Phangnga, 9 genotypes from Krabi, 12 genotypes from Surat Thani, 10 genotypes from Trang and 11 genotypes from Chumphon). The rest 48 genotypes were grouped together in Group 2 (10 genotypes from Phangnga, 11 genotypes from Krabi, 8 genotypes from Surat Thani, 10 genotypes from Trang and 9 genotypes from Chumphon).

The PCoA based on SSR results also clustered the oil palm genotypes into 2 groups (Figure 2) with identical genotype members in each group. The PCo1 and PCo2 explained 21.28% and 16.41% of variation, respectively. It is possible that these two groups of oil palm genotypes are seedlings of *tenera* variety which were produced from different commercial seed companies. In Thailand, *tenera* planting materials were distributed by few companies possessing their own breeding populations. Though the genetic materials utilized for producing commercial oil palm varieties in Indonesia, Malaysia and Thailand were mostly derived from four Dura plants grown at Bogor Botanical Garden, Indonesia in 1848 (Basiron, 2002; Corley and Tinker, 2003) indicating narrow genetic background, the considerable genetic variation among breeding populations of commercial companies has been reported (Taeprayoon *et al.*, 2015). Similar situation was observed in other major oil palm growing and supplying countries. Arias *et al*., (2012) studied genetic similarity among commercial oil palm from Malaysia, France, Costa Rica and Colombia using SSR markers and found that commercial oil palm individuals were clustered into two groups reflecting base breeding populations and selection methods. According to the results of the present study, oil palm genotypes should be taken from two, rather than one cluster to capture and maximize genetic variability for breeding population.

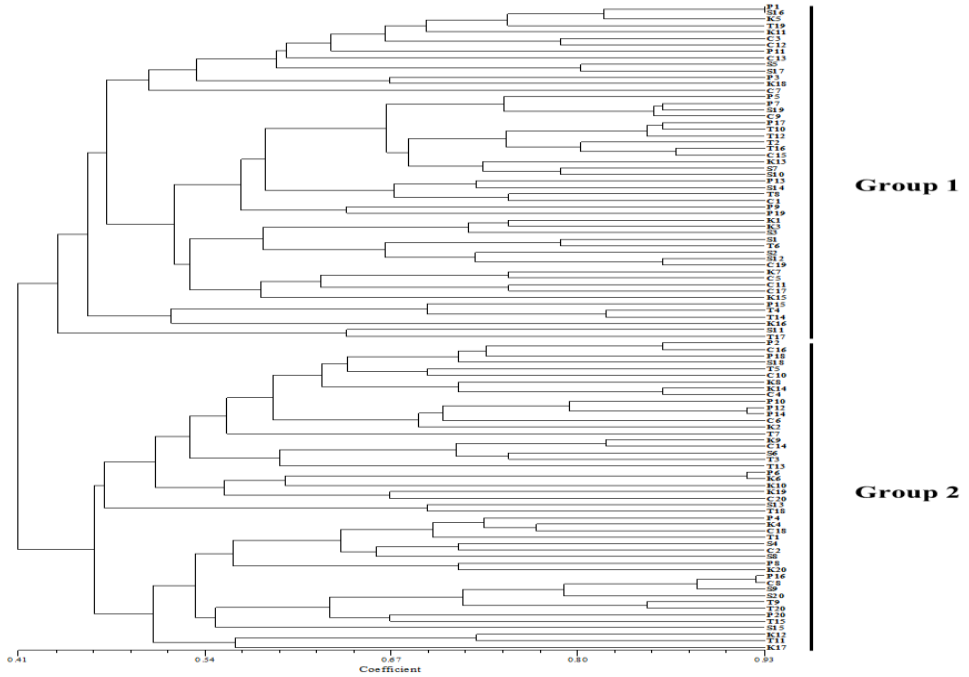

Figure 1: UPGMA cluster analysis of 100 oil palm genotypes using the Jaccard's similarity coefficient

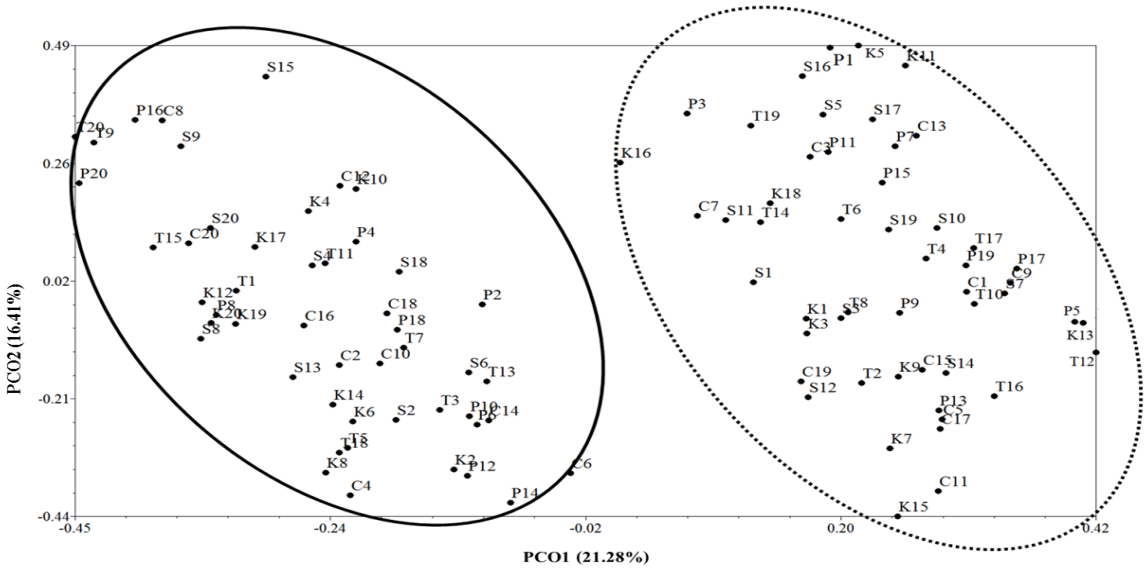

Figure 2 Principal coordinates analysis (PCoA*)* based on Jaccard's similarity coefficient of 100 oil palm genotypes

CONCLUSION

It is undoubted that oil palm breeding programs directed by private and public organizations have brought a success of high yielding oil palm plantation through superior varieties. However, under this changing environment which bring in adverse biotic and abiotic stresses such as salinity and drought, oil palm breeding program must continue to develop new varieties with high level of stability and adaptability. Genetic variability of the base populations is essential element for successful breeding program. This study on molecular characterization would be very helpful for oil palm breeders to select genotypes better and plan an effective breeding scheme. A total of 100 genotypes collected from different provinces in southern Thailand did not show population structure based on sampling locations. However, seven SSR primers used successfully differentiated and clustered the collected oil palms. UPGMA cluster analysis and PCoA analysis separated the oil palm genotypes into two major groups. Therefore, genotypes to be included for breeding population and the crosses of parental oil palm should be the genotypes from two different groups to maximize genetic variability.

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