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Genetic background of three commercial oil palm breeding populations in Thailand revealed by SSR markers

Puntaree Taeprayoon[1], Patcharin Tanya[2*], Suk-Ha Lee[3,4] and Peerasak Srinives[2]

[1]Program in Plant Breeding, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen, Nakhon Pathom 73140, Thailand
[2]Department of Agronomy, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen, Nakhon Pathom 73140, Thailand
[3]Department of Plant Science and Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Korea
[4]Plant Genomics and Breeding Institute, Seoul National University, Seoul 151-921, Korea

[*]Corresponding author: altanya55@yahoo.com

Abstract

A total of 121 oil palm (*Eleais guineensis* Jacq.) breeding plants from three populations designated as P1, P2 and P3 were obtained from three breeding companies located in three provinces of Thailand, viz. Prachuap Khiri Khan, Kanchanaburi and Krabi, respectively. Although, these populations came from different sources, they share some common genetic backgrounds of Deli Dura and AVROS Pisifera which are the main germplasm sources of commercial oil palm breeding programs in Thailand. All plants were assessed by 96 SSR markers from which 20 of them were polymorphic giving 109 alleles with polymorphic information contents ranged from 0.45 to 0.87 for mEgCIR580 and mEgCIR787 markers, respectively. The number of alleles per locus ranged from 3 to 8 with averages of 5.05, 3.80 and 3.75 for P1, P2 and P3 and the overall average of 5.45. An analysis of molecular variance (AMOVA) gave variation among populations and individuals within populations at 33 % and 67 %, respectively. Nei's genetic distance showed that variability among populations were 0.53 (P1 vs. P3), 0.61 (P1 vs. P2) and 0.62 (P2 vs. P3). A dendrogram constructed from Jaccard's similarity coefficients of marker variation separated the oil palm trees into 4 clusters following their respective breeding companies.

Keywords: DNA fingerprinting, *Eleais guineensis*, Simple sequence repeat, Microsatellite, Genetic variation.
Abbreviations: A_e_effective number of alleles per locus, FFB_fresh fruit bunch, F_{IS}_inbreeding coefficient, F_{ST}_Wright's fixation index, G_{st}_inter-population genetic diversity differentiation, H_e_expected heterozygosity, H_S_intra-population genetic diversity, H_T_average total genetic diversity of population, *I*_Shannon's information index, N_m_average gene flow, OER_oil extraction rate, PCA_principal component analysis, PIC_polymorphism information content, SSR_simple sequence repeat.

Introduction

Oil palm (*Elaeis guineensis* Jaqc.) is the most important oil crop giving the highest yield per hectare among all oil crops in the world (MPOC, 2008). Palm oil and its derived products are used in several industries including food, feed, fuel and cosmetic. Indonesia and Malaysia are the leading producers and exporters, while Thailand, although far behind is the third. The planted area in Thailand in 2013 was 636,800 ha with a national plan to increase to 1.6 million ha in 2029 following the policy of Ministry of Energy in using palm oil as the main raw material for biodiesel production. Thus it is essential to improve oil palm germplasm in Thailand for increasing oil extraction rate (OER) of fresh fruit bunch (FFB), slow trunk growth, resistance to diseases and pests with other desirable agronomic traits (Office of Agricultural Economics, 2013). Most oil palm cultivars are of Tenera fruit type produced from crosses between Dura type (female parent) and Pisifera type (male parent). The Dura type sets fruits with a thick shell (2-8 mm) amounts to 25-55 % w/w while the mesocarp amounts to 35-70 % w/w of fruit. The Pisifera type has a very thin shell or shell-less with 83-99 % w/w of mesocarp, whereas the Tenera type has a thin shell (0.5-4 mm) comprising 5-30 % w/w and mesocarp comprises 60-91 % w/w of fruit. Genetic background of breeding materials is an essential foundation for a breeding gain. Nowadays, molecular markers are indispensable tools for determining genetic variation in plant germplasm and fingerprinting of plant cultivars. Most commercial oil palm cultivars grown in Indonesia, Malaysia and Thailand were derived from four Dura plants grown at Bogor Botanical Garden, Indonesia in 1884 (Corley and Tinker, 2003). This indicates that the genetic background of commercial oil palm is very narrow. To increase the genetic variability in a breeding program, more parental stocks should be made available (Bakoumé and Louise, 2007). Oil palm cultivars are currently developed using conventional breeding and selection which normally takes 10-15 years per cycle. Breeding strategies are aimed to producing Dura × Pisifera hybrid (called Tenera type) with high oil yield performance. Genetic difference of the parents is one of the most important criteria to produce a superior hybrid and

increase the efficiency of the breeding programs (Cochard et al., 2009). Molecular markers can be employed to assess genetic variability and help select the suitable parents for production of a new hybrid. Various molecular markers are being applied for oil palm improvement (Prize et al., 2007; Rival, 2007). Microsatellite or Simple Sequence Repeats (SSR) are considered the most useful markers because they are performed based on polymerase chain reaction (PCR), simple, highly polymorphic, dispersed throughout the genome and co-dominant. Oil palm SSR markers had been developed, characterized and tested across-taxa by Billotte et al. (2001). The markers were later used for constructing a genetic linkage map (Billotte et al., 2005), detecting quantitative trait loci (Billotte et al., 2010), and assessing genetic variation and diversity (Putri et al., 2010). SSR markers were also developed and used in other palm species such as *E. oleifera* (Zaki et al., 2012), *Phoenix dactylifera* L. (Bodian et al., 2012), and *Cocos nucifera* L. (Konan et al., 2011). The information on genetic diversity background is advantageous to maintain and manage the oil palm germplasm *ex situ* that reduces cost and area. The objective of this study was to investigate genetic variation in commercial oil palm populations from three major breeding companies in Thailand using microsatellite markers.

Results

Genetic variability

Out of the 96 SSR markers used in this study, 20 were polymorphic giving a total of 109 alleles (Table 2). Each primer produced from 3 alleles (mEgCIR146) to 10 alleles (mEgCIR787) with an average of 5.45±1.85 alleles. The observed number of alleles per locus ranged from 3 to 8 in P1, 2 to 9 in P2, and 2 to 7 in P3. While the allele means ranged from 3 (mEgCIR146, mEgCIR580 and mEgCIR783) to 7 (mEgCIR801), with an average of 4.20±1.06 alleles. The mean number of alleles per locus were 5.05±1.50, 3.80±1.61, and 3.75±1.48 for P1, P2 and P3, respectively. The PIC values ranged from 0.45 to 0.88 with an average of 0.65. The lowest and highest PIC values were obtained from mEgCIR580 (0.45) and mEgCIR787 (0.88), respectively. Single-population statistical analysis revealed that effective number of alleles per locus (A_e) in each population were 2.95±1.01 alleles in P1, 2.67±1.28 alleles in P2, 2.42±0.79 alleles in P3 with an average of 2.68±1.03 alleles. Heterozygosity (H_e) in each population were 0.62±0.15 in P1, 0.57±0.16 in P2 and 0.54±0.15 in P3. Averaged across 3 populations, *I* and F_{IS} were 1.05±0.36 and 0.20, respectively. While multi-population statistical analysis gave an A_e of 3.76±1.52 alleles and H_e of 0.70±0.10, indicated a high level of gene diversity while, *I* and F_{IS} were 1.40±0.33, and 0.22, respectively (Table 3).

Allele frequencies

Eighteen of the 20 amplifiable SSR markers produced a total of 109 alleles of which 47 (43.12 %) of them were specific alleles. The total number of specific alleles in P1, P2 and P3 were 39 (57.35 %), 16 (23.53 %) and 13 (19.12 %), respectively (Table 4). Nineteen alleles (40.43 %) were specific to P1, 4 (8.51 %) specific to P2, 3 (6.38 %) specific to P3, 11 (23.40 %) specific to P1 and P2, 9 (19.15 %) specific to P1 and P3 and 1 (2.13 %) specific to P2 and P3. Specific alleles that distinguished P1 from the other groups were alleles 4 to 7 of mEgCIR195, alleles 4 and 5 of mEgCIR243, allele 4 of mEgCIR425, alleles 3 to 5 of mEgCIR580, alleles 4 and 5 of mEgCIR786, alleles 6 to 10 of mEgCIR787, allele 8 of mEgCIR790 and allele 4 of mEgCIR795. Specific alleles found only in P2 were allele 4 of mEgCIR783, allele 4 of mEgCIR787, and alleles 8 and 9 of mEgCIR801. The markers that separated P3 from the other populations were allele 7 of mEgCIR774, allele 5 of mEgCIR775 and allele 5 of mEgCIR800 (Table 4).

Genetic relatedness

A dendrogram and principal component analysis (PCA) separated the breeding populations into 4 clusters with a high cophenetic correlation (r) of 0.90 (Fig 1), implying that the clustering is reliable. Cluster A comprises the breeding materials derived from open pollination of P1 company from Prachuap Khiri Khan province, Cluster B contains the materials from P2 population from Kanchanaburi province, while Cluster C contains P3 materials of the company in Krabi province. Cluster B and C include plants of all 3 fruit types (Dura, Pisifera and Tenera), while Cluster D contains the plants of Pisifera and Tenera fruit types from P1. The genetic distances (Nei, 1978) between groups were 0.61 (P1 and P2), 0.53 (P1 and P3) and 0.62 (P2 and P3). The biplot of principal coordinate analysis also confirmed the separation power of the same four groups (Fig 2). The AMOVA in Table 5 show 33 % genetic variation among the breeding materials of the three companies and 67 % among plants of the same populations. This confirms a moderately high variation in breeding materials used by the oil palm companies. The estimates of genetic variation from AMOVA led to calculation of H_T, H_S, G_{st}, N_m and F_{ST} at 21.73, 7.14, 0.67, 0.51 and 0.33, respectively.

Discussion

The percentage of amplified bands in P1, P2 and P3 were 92.66 %, 69.72 % and 68.81 %, respectively. P1 showed the highest PIC value and A_e at 0.57 and 2.95, respectively, while P3 was the lowest in the same parameters (0.48 and 2.42, respectively). This revealed that the breeding stock of P1 company is the most diverse, this is because the materials from P1 have been exposed to less selection pressure than those from the other companies. PIC for locus mEgCIR787 showed the highest value, and thus it is suitable for application in marker-assisted breeding programs. The number of alleles per locus in our study varied from 3-10 alleles which were similar to that reported by Putri et al. (2010). They found 3-13 alleles each among 20 SSR loci used in studying diversity of Pisifera oil palm germplasm of Sampoerna Agro Company. The germplasm originated from the oil palm populations of Dami, Ekona, Ghana, Nigeria and Yangambi. Their genetic variation was drastically reduced after several cycles of selection. In our study, an average of SSR alleles per locus was 5.45 and effective number of alleles per locus (A_e) was 2.68 alleles. While, Hayati et al. (2004) studied isozyme variation in African oil palm germplasm and found 1.43-2.14 alleles/locus with a mean of 1.80 alleles. Maizura et al. (2006) assessed the oil palm germplasm from 11 African countries and detected 1.3-1.9 alleles per RFLP locus. Decreasing in number of alleles per locus is a clue to the loss of genetic variability in the breeding population. The A_e obtained in our experiment (2.42 to 2.95) is higher than those previously reported by Hayati et al. 2004 (1.17 to 1.55) and Maizura et al. 2006 (1.5 to 1.9). The SSR markers are more stable and

Table 1. Oil palm samples of 121 plants randomed from 3 breeding populations and used in this study. The plant number assigned to each plant code samples is indicated in the brackets.

Source	Plant code			Total no. of plants
	Dura	Pisifera	Tenera	
P1 (Prachuap Khiri Khan province)	BD (1-53)	BP (1)	BT (1-13)	67
P2 (Kanchanaburi province)	UD (1-9)	UP (1-10)	UT (1-8)	27
P3 (Krabi province)	KD (1-12)	KP (1-7)	KT (1-8)	27
Total	74	18	29	121

P1 : Deli Dura and the progenies derived from Deli Dura × AVROS Pisifera, P2 : Progenies of Deli Dura × Semi Dumpy AVROS Pisifera, P3 : Progenies of Deli Dura × Dumpy AVROS Pisifera.

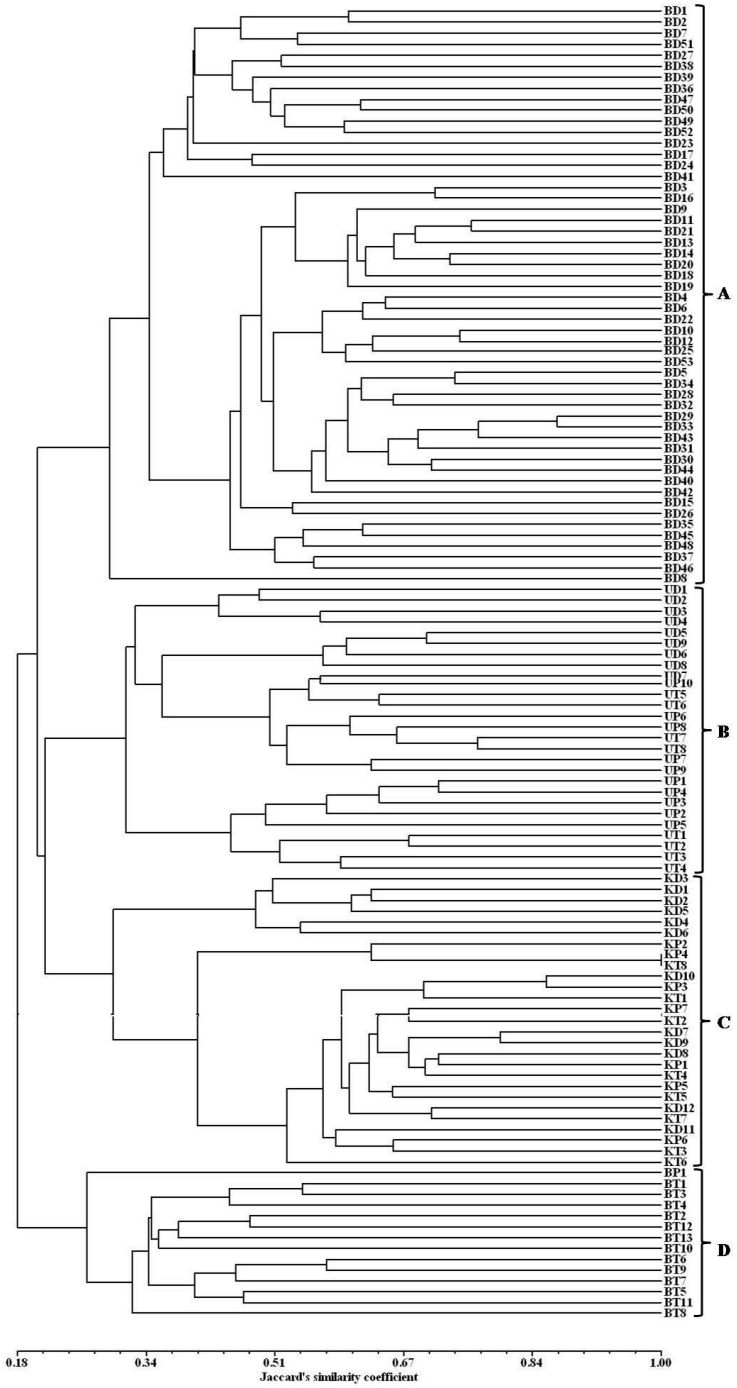

Fig 1. A dendrogram of Jaccard similarity's coefficients as determined from similarity among the SSR markers of oil palm. Cluster A comprises the Dura of P1 population, cluster B contains plants of P2, cluster C contains samples of P3, while cluster D includes plants of Pisifera and Tenera types from P1.

Table 2. Allele number and PIC value obtained from 20 SSR markers being amplified on 3 oil palm breeding populations.

Locus	Repeat motif	Linkage group	Annealing temp. (°C)	No. of alleles	No. of alleles in each population			Allele mean	PIC
					P1	P2	P3		
mEgCIR037	$(GA)_{17}$	15	52	5	5	5	4	4.66	0.64
mEgCIR146	$(GT)_2(GA)_{27}$	10	58	3	3	3	3	3.00	0.49
mEgCIR195	$(GA)_{21}$	6	58	7	7	3	3	4.33	0.61
mEgCIR243	$(GA)_{17}$	10	52	5	5	3	2	3.33	0.66
mEgCIR380	$(CT)_8(AT)_3(GT)_{11}$	1	52	5	5	3	2	3.33	0.69
mEgCIR425	$(CCG)_9$	3	58	4	4	3	3	3.33	0.61
mEgCIR521	$(GA)_{19}$	15	52	4	4	4	4	4.00	0.53
mEgCIR551	$(GA)_{18}$	10	52	6	6	4	6	5.33	0.74
mEgCIR580	$(GA)_{10}$	6	52	5	5	2	2	3.00	0.45
mEgCIR774	$(GA)_{20}$	8	52	7	6	6	6	6.00	0.77
mEgCIR775	$(GA)_{13}$	8	52	5	4	3	5	4.00	0.62
mEgCIR778	$(CA)_6(GA)_{14}$	8	52	4	4	4	3	3.66	0.70
mEgCIR781	$(GA)_{17}$	15	52	4	4	2	4	3.33	0.54
mEgCIR783	$(GA)_{15}$	6	58	4	3	3	3	3.00	0.58
mEgCIR786	$(GA)_{16}$	4	52	5	5	3	2	3.33	0.70
mEgCIR787	$(GA)_{13}$	15	52	10	8	5	3	5.33	0.88
mEgCIR790	$(GA)_{19}$	12	52	8	8	5	7	6.66	0.80
mEgCIR795	$(CA)_6(GA)_{20}$	4	56	4	4	3	3	3.33	0.61
mEgCIR800	$(GA)_{18}$	2	56	5	4	3	5	4.00	0.61
mEgCIR801	$(GA)_{22}$	4	56	9	7	9	5	7.00	0.82
Total				109	101	76	75		
Mean			53.8	5.45	5.05	3.80	3.75	4.20	0.65
±SD			±2.59	±1.85	±1.50	±1.61	±1.48	±1.06	±0.11
PIC					0.56	0.51	0.47		

PIC : polymorphism information content

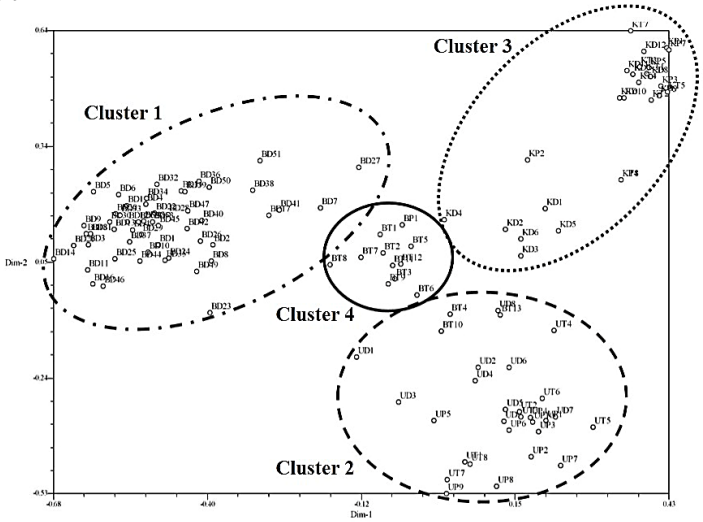

Fig 2. Principal component analysis using Jaccard's similarity coefficients based on the SSR markers of oil palm collections showing cluster 1 (Dura type of P1), cluster 2 (Dura, Pisifera and Tenera types of P2), cluster 3 (Dura, Pisifera and Tenera types of P3) and cluster 4 (Pisifera and Tenera types of P1).

distributing throughout the genome as compared to isoenzyme and RFLP markers. Specific alleles found in each population can be used to develop markers for selection of gene/QTL of elite parents to produce superior hybrids. Similar studies were reported in oil palm and other plants in the same Arecaceae family. Perera et al. (2000) used 8 SSR markers to determine genetic diversity and population structure in 75 tall and 55 dwarf coconuts (*Cocos nucifera*) plants. They found 24 and 1 specific alleles for classifying tall and dwarf plants, respectively. Moretzsohn et al. (2002) used 96 RAPD primers to study genetic diversity in 175 Brazilian oil palms (*E. oleifera*) and 17 accessions of African oil palm. They found 14 primers specific to *E. guineensis* and 12 specific to *E. oleifera*.

Expected heterozygosity (H_e) in P1, P2 and P3 were 0.62, 0.57 and 0.54, respectively, revealing that these breeding populations still maintain high heterozygosity between plants within the populations, probably due to mild selection imposed on them. P2 and P3 revealed similar A_e, H_e and *I*. Both populations were derived from a few plants of Ulu Remis Dura x AVROS Pisifera and being used in many breeding programs in the world (Corley and Tinker, 2003). P3 showed low F_{IS} value (0.08) or high heterozygosity of plants in this population, while P1 and P2 gave higher F_{IS} at 0.27 and 0.25, respectively. Hayati et al. (2004) also found low F_{IS} value of 0.088 in 86 Tanzanian oil palm. The variation among populations (33 %) in our study was moderately high implying that these breeding stocks were rather different.

Table 3. Genetic variability parameters of 3 oil palm groups based on 20 SSR markers.

Group	Sample size	PIC± SD	A_e ± SD	H_e ± SD	*I* ± SD	F_{IS}
Single-population description statistics						
P1	67	0.57±0.15	2.95±1.01	0.62±0.15	1.18±0.33	0.27
P2	27	0.51±0.16	2.67±1.28	0.57±0.16	1.02±0.39	0.25
P3	27	0.48±0.15	2.42±0.79	0.54±0.15	0.96±0.34	0.08
Mean			2.68±1.03	0.58±0.15	1.05±0.36	0.20
Multi-population description statistics						
			3.76±1.52	0.70±0.10	1.40±0.33	0.22

P1 : Deli Dura and the progenies derived from Deli Dura × AVROS Pisifera, P2 : Progenies of Deli Dura × Semi Dumpy AVROS Pisifera, P3 : Progenies of Ulu Remis Dura × Dumpy AVROS Pisifera. PIC : polymorphism information content, A_e : effective number of alleles per locus, H_e : expected heterozygosity , *I* : Shannon's information index, F_{IS} : inbreeding coefficient.

Table 4. Allele frequency and size (bp) of 18 SSR loci with specific alleles in 3 oil palm breeding populations showing presence (+) or absence (-) of the alleles.

No.	Locus	Allele order	Allele frequency	Size (bp)	Present/absent in population		
					P1	P2	P3
1	mEgCIR037	5	0.149	173	+	+	-
2	mEgCIR195	4	0.017	213	+	-	-
		5	0.054	210	+	-	-
		6	0.004	206	+	-	-
		7	0.004	205	+	-	-
3	mEgCIR243	3	0.186	228	+	+	-
		4	0.004	226	+	-	-
		5	0.145	224	+	-	-
4	mEgCIR380	2	0.244	186	+	-	+
		3	0.302	180	+	+	-
		4	0.289	177	+	+	-
		5	0.008	175	+	+	-
5	mEgCIR425	4	0.058	257	+	-	-
6	mEgCIR551	5	0.157	222	+	-	+
		6	0.223	216	+	-	+
7	mEgCIR580	3	0.054	243	+	-	-
		4	0.045	241	+	-	-
		5	0.008	235	+	-	-
8	mEgCIR774	6	0.045	159	+	+	-
		7	0.070	155	-	-	+
9	mEgCIR775	4	0.095	238	+	-	+
		5	0.004	236	-	-	+
10	mEgCIR778	4	0.207	182	+	+	-
11	mEgCIR781	3	0.587	150	+	-	+
		4	0.066	148	+	-	+
12	mEgCIR783	1	0.149	337	+	-	+
		4	0.045	311	-	+	-
13	mEgCIR786	3	0.244	275	+	+	-
		4	0.186	270	+	-	-
		5	0.050	264	+	-	-
14	mEgCIR787	2	0.087	178	-	+	+
		4	0.079	173	-	+	-
		5	0.079	171	+	+	-
		6	0.161	167	+	-	-
		7	0.017	151	+	-	-
		8	0.062	146	+	-	-
		9	0.107	135	+	-	-
		10	0.124	116	+	-	-
15	mEgCIR790	6	0.054	216	+	-	+
		8	0.157	209	+	-	-
16	mEgCIR795	4	0.413	180	+	-	-
17	mEgCIR800	4	0.099	215	+	-	+
		5	0.021	212	-	-	+
18	mEgCIR801	4	0.083	210	+	+	-
		7	0.244	190	+	+	-
		8	0.021	186	-	+	-
		9	0.017	178	-	+	-
	Total	47			39	16	13

Table 5. AMOVA of data from 20 SSR markers applied to 121 plants of three commercial oil palm breeding populations.

Source	df	MS	EMS	Est. var.	% of variation
Among populations	2	271.0**	$\sigma^2_w + k\sigma^2_i$	7.14	33
Within populations	118	14.6	σ^2_w	14.59	67
Total	120			21.73	100

** Significant at $P \leq 0.01$, σ^2_w: variance within populations, σ^2_i: variance among populations, k = 35.93 (estimated number of samples in each populations).

However, high genetic variation among plants of the same population revealed that each company was still carrying a diverse genetic stock for its breeding program. Futuyama (1986) reported an equation to calculate the rate of loss in heterozygosity at approximately 1/2N per generation, when N is the population size. This statement was confirmed by the combined data of all 121 plants in a multi-population analysis that gave H_e of 0.70, which higher than a single-population mean. This gives a clue that if these companies share their germplasm, they can have a pool of oil palm plants with higher genetic variation for selection in their breeding programs. The H_e values also reflected different level of genetic diversity found in the breeding populations. Hayati et al. (2004) found H_e = 0.18 in *E. guineensis*, Wickneswari et al. (1997) found H_e = 0.10 in *Calamus manan* Miq. (manan rattan palm), Eguiarte et al. (1992) found H_e = 0.15 in *Astrocaryum mexicanum* Liebm. ex. Mart. (chocho palm), Shapcott (1998) found H_e = 0.14 in *Carpentaria acuminate* (carpentaria palm), Zehdi et al. (2004) found H_e = 0.70 in *Phoenix dactylifera* L. (date palm), and Gaiotto et al. (2003) found H_e = 0.73 to 0.79 in *Euterpe edulis* Mart. (heart-of-palm). As expected, they explained that the populations which were subjected to more selection cycles generally had lower H_e. Shah et al. (1994) determined Shannon's index (*I*) in oil palm using RAPD markers and found 0.21 to 0.43 in 8 provenances in Africa. While our study showed the *I* values of 1.18 in P1, 1.02 in P2, and 0.96 in P3 with the average of 1.05. Thus, this Thai oil palm germplasm showed higher genetic variation than the African oil palm. However, this difference can be due to the fact that SSR markers give higher variation than RAPD. Our genetic differentiation (F_{ST}) among 3 populations was 0.33 indicated high genetic divergence which was supported by highly significant difference of F-test (F = 18.57, P = 0.01) of the AMOVA in Table 5. The average gene flow (N_m) was 0.51 indicated a high level of common DNA fragments. The H_T and H_S values were 21.73 and 7.14 (Table 5) so the inter-population diversity was high as confirmed by G_{st}. Our G_{st} was 0.67 compared to the previous research by Zehdi et al. (2004) in *Phoenix dactylifera* L. (Tunisian date) (G_{st} = 0.06), by Adin et al. (2004) in *Bactris gasipaes* Kunth. (peach palm) (G_{st} = 0.03-0.04), and by Gaiotto et al. (2003) in *Euterpe edulis* Mart. (heart of palm) (G_{st} = 0.07). The dendrogram in Fig 1 separated plants in population P1 into two clusters, A and D. Cluster A comprises 53 Dura plants while cluster D comprises one Pisifera and 13 Tenera plants. This company obviously stocks its germplasm from two different populations. Thus plants of P1 showed higher genetic variation than those of P2 and P3. P2 and P3 were distinctly clustered into groups B and C, respectively.

Materials and Methods

Plant materials

Leaf samples were collected from 121 oil palm plants covering 3 fruit types (Dura, Pisifera and Tenera) from 3 Thailand's most active breeding companies located in 3 provinces, viz. Prachuap Khiri Khan, Kanchanaburi and Krabi (Table 1). These companies have their own oil palm breeding programs and produced oil palm hybrid seeds for growers. The first population (P1) comprised 67 plants of 53 Dura (BD 1-53), one Pisifera (BP 1) and 13 Tenera (BT 1-13) of one commercial company at Bang Saphan Noi district, Prachuap Khiri Khan province, lower central Thailand. The origin of BD was Deli Dura while BP and BT were selected palms from the farmer plantation in the same province and derived from Deli Dura × AVROS Pisifera. The second population (P2) comprised 27 plants of nine Dura (UD 1-9), 10 Pisifera (UP 1-10) and eight Tenera (UT 1-8) of another commercial company at Thong Pha Phum district, Kanchanaburi province, western Thailand. This population was derived from Deli Dura × Semi Dumpy AVROS Pisifera. The third population (P3) comprised 27 plants of 12 Dura (KD 1-12), seven Pisifera (KP 1-7) and eight Tenera (KT 1-8) of the third breeding company grown at Mueang district, Krabi province, southern Thailand. They were developed from the progenies of Deli Dura × Dumpy AVROS Pisifera.

PCR amplification

Genomic DNA of the oil palm was extracted from young leaves following the method of Tanya et al. (2011). Ninety-six SSR markers were chosen from 16 linkage groups of the oil palm as reported by Billotte et al. (2005). The total PCR reaction (10 µl) required 10 ng/µl of genomic DNA in 1x *Taq* buffer with $(NH_4)_2SO_4$ (Fermentas), 2 mM $MgCl_2$, 10 mM dNTP of each nucleotide, 5 µM each of forward and reverse primers and 1 U *Taq* DNA polymerase. Thirty-five cycles of PCR were performed in a PCT-100^{TM} Thermal Controller (MJ Research, Watertown, MA, USA). In each cycle, there were 5 min pre-denaturing at 94 ºC, 30 s denaturing at 94 ºC, 30 s annealing and 1 min extension at 72 ºC. The SSR-PCR products were detected in 5 % polyacrylamide gel and stained with silver nitrate.

Data analysis

Data were recorded as presence (1) or absence (0) of the band alleles of each locus and used in evaluating polymorphism information content (PIC) using the formula $PIC_i = 1 - \sum p_i^2$, where p_i is the frequency of the i^{th} allele of each locus. POPGENE Version 1.31 (Yeh et al., 1999) was employed to determine mean number of alleles, effective number of alleles per locus (A_e), Shannon's information index (*I*) (Lewontin, 1972), and expected heterozygosity (H_e). An analysis of molecular variance (AMOVA) and estimated number of samples in each populations (*k*) were carried out by GENALEX version 6.3 (Peakall and Smouse, 2006) to explain genetic variability among and within the oil palm populations. Their parameters were average total genetic diversity of population (H_T), intra-population genetic diversity (H_S), and inter-population genetic diversity differentiation (G_{st}). These parameters explain gene

differentiation for the observed loci in each population as well as in the combined population. Inbreeding coefficient (F_{IS}) was calculated to measure the deviation of genotypic frequencies from Hardy-Weinberg Equilibrium within single-population, while F_{ST} (Wright's fixation index) was used to indicate gene differentiation between populations. The average gene flow (N_m) among the populations was calculated from the equation $N_m = 0.25 (1-F_{ST})/F_{ST}$ (Slatkin and Barton, 1989) using F-statistic (Wright, 1978). NTSYSpc software version 2.20e (Rohlf and Sokal, 1981) was used to perform a principal component analysis (PCA) to determine the genetic relationship among individuals. To cluster the samples, an unweighted neighbor-joining tree was constructed based on Jaccard's similarity coefficient.

Conclusion

The SSR markers used in this study were able to classify the genetic background of major oil palm genetic stocks in Thailand. All three companies in this study have maintained high variation among plants, thus crossing between plants within each population should be able to promote superior hybrids in their breeding programs. An exchange of germplasm among the companies is encouraged to make full use of the oil palm genetic variation.

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