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Full Paper

Assessment of genetic relationships among pummelo cultivars [*Citrus maxima* (Burm.) Merrill] using simple sequence repeat markers

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Abstract: The genetic relationships among Thai pummelo cultivars were estimated using simple sequence repeat (SSR) markers developed for *Citrus* spp. Fifty three clones, including outstanding commercial cultivars, local cultivars from northern, central and southern provinces of Thailand and foreign cultivars were evaluated using 13 SSR markers. The results show that 10 SSR markers are polymorphic and generate a total of 33 alleles. The average value of the expected heterozygosity and polymorphism information content are 0.52 and 0.45 respectively, and the combined probability of the identity value of the 10 SSR markers is 6.82×10^{-6}. The similarity index (SI) ranges from 0.44 to 1.0. The pummelos can be clustered into seven groups and one out-group by unweighted pair group method with arithmetic averages. Most pummelos including commercial cultivars and hybrids of 'Thong Dee' and of 'Ta Khoy' are classified in the same group (SI = 0.67-1.00), whereas local and foreign cultivars are more diverse than commercial cultivars (SI = 0.44).

Keywords: pummelo, *Citrus maxima*, genetic diversity, microsatellite marker, cluster analysis, principle coordinate analysis

INTRODUCTION

The pummelo is a tropical fruit native to the Malay Peninsula and Thailand, and from here it has been introduced to India and China [1]. Thailand has the highest number of pummelo cultivars in the world and there is considerable genetic variation among the cultivars [2]. Pummelo is largely self-incompatible [3, 4] and unlike other *Citrus* species, it does not produce nucellar seedlings. Hybridisation can occasionally occur with other species of the genus.

Maejo Int. J. Sci. Technol. **2016**, *10*(02), 209-219

Pummelo is an economic fruit crop of Thailand with an increasing export demand. Its export value in 2014 was 227 million baht to markets such as Hong Kong, Canada, China and Singapore [5]. Production problems of pummelo in Thailand include low yield of quality fruit and high dependence on pesticides. To obtain a sustainable strategy for their production these problems may be overcome by developing new cultivars that present high yields and greater resistance to pests and diseases. At present, all prominent cultivars are chance-seedlings with neither reliable pedigrees nor genetic information. In addition, several varieties of pummelo possess similar morphological traits but have been given different names in different areas, resulting in an ambiguity for a large number of names [6, 7]. Sethpakdee [2] concluded that two important commercial cultivars, 'Kao Numphueng' and 'Kao Tanggua', were a single clone. Genetic information of pummelo cultivars would be helpful in eliminating this confusion and provide plant breeders with a more suitable breeding strategy. The genetic information of pummelo is very limited as most research has only focused on morphological traits in commercial cultivars. Cultivar identification based only on morphological traits is difficult [8]; therefore, germplasm assessment of the genetic relationship of both commercial and local cultivars is an important step in developing a new, improved cultivar.

The use of molecular markers has become a reliable method for genetic diversity assessment. Molecular makers are a powerful, accurate, and rapid technique for analysing and comparing DNA fingerprints in plants because DNA is specific to each species**,** detectable in all tissues and independent of environmental variation [9]. Molecular marker technologies could help to estimate and classify relationships of perennial fruit germplasm, including cultivar identification, genetic diversity and germplasm management [9-12].

Simple sequence repeat (SSR) markers, also known as micro satellites, have been commonly used to examine genetic variation. They are useful because they are based on polymerase chain reactions (PCR), highly reproducible and polymorphic, generally co-dominant markers having abundant loci in the plant genome and a comprehensive genome coverage, and require only a small amount of starting DNA for replication [13, 14].

Several molecular markers have been used to measure the genetic relationships and diversity in citrus germplasm, including random amplification of polymorphic DNA, amplified fragment length polymorphism (AFLP), inter simple sequence repeat and SSR. For example, Roose et al. [16] used restriction fragment length polymorphism (RFLP) to study the genetic diversity of 59 pummelo, 24 citron and 48 trifoliate orange accessions of the citrus collection of University of California. They reported that pummelo presents great polymorphism and a high level of heterozygosity while citron and trifoliate oranges are almost monomorphic. Kijas et al. [16] used microsatellite and RFLP markers to construct a genetic map with different linkage groups of *Citrus*. The markers were developed by using the DNA sequence of a hybrid between rangpur lime and trifoliate orange. Ahmad et al. [9] developed 26 microsatellite markers from 'Washington Navel' *Citrus*. These SSR markers can discriminate sweet orange, mandarin, grapefruit, lemon and citrange. Barkley et al. [10] estimated a genetic diversity of 370 *Citrus* accessions maintained at University of California with 24 SSR markers, and some of these markers were developed from genomic libraries of pummelo in California. In the pummelo section, 76 pummelos and 13 pummelo hybrids were shown to have a moderate level of polymorphism with an average of the expected heterozygosity (He) value of 0.42.

On the other hand, Yong et al. [17] found a low level of polymorphism with an average He value of 0.25 for 110 pummelo cultivars in China using AFLP and SSR markers. Liang et al. [18] studied the transcriptome assembly of pummelo and the genetic relationship between pummelo and

Maejo Int. J. Sci. Technol. **2016**, *10*(02), 209-219

other *Citrus* species. They developed 1,174 SSR loci from the DNA sequencing of sweet orange (*Citrus sinensis)*. Of these primers, only 29 were useful for phylogenetic analysis of *Citrus* species including *C. grandis*, *C. paradise*, *C. sinensis*, *C. aurantium*, *C. ichangensis*, *C. reticulata*, *C. aurantifolia*, *C. limon*, *C. medica*, *C. jambhiri* and *C. hongheensis*. Chai et al. [19] used expressed sequence tag (EST) – SSR markers to evaluate the polymorphism of pummelo and its relative species. The genetic relationship between pummelo and its relatives, determined by the EST microsatellite of *C. grandis*, was consistent with the previous *Citrus* taxonomy. Nevertheless, almost all molecular markers evaluated for genetic diversity of pummelo have been developed from genomic libraries of other *Citrus* species, and few markers have been established from genomic libraries of pummelo [18]. In Thailand molecular studies of pummelos on genetic diversity and examination of their relationships have been limited. The objective of this study is to evaluate the genetic relationship of pummelo cultivars in Thailand using SSR markers.

MATERIALS AND METHODS

Pummelo Samples, DNA Extraction and SSR Markers

Fifty-three pummelo samples comprising commercial and local cultivars from northern, central and southern Thailand, and foreign cultivars from the US as well as one grapefruit, were used in this study (Table 1). Young and clean fresh leaf samples were used for DNA extraction.

Commercial cultivars are those that are well known and grown widely throughout Thailand. Local cultivars are those grown in a limited area or particular provinces. They are not very well known to most consumers or are available to only a limited market. Foreign cultivars are those commercialised in other countries but not in Thailand.

The total DNA was extracted using the cetyltrimethylammonium bromide method [20]. DNA concentration and quality were measured using a UV spectrophotometer (Biomate, USA). Thirteen SSR markers consisted of 3 groups: (1) P73, P620 and P1826 markers [11], (2) CAC23 and TAA41 markers [16], and (3) AC01, AG14, CMS4, CMS24, CT02, CT19, CT21 and GT03 markers [10]. These markers were developed from *Citrus* spp and were selected to evaluate their diversity. Six markers, viz. AC01, AG14, CT02, CT19, CT21 and GT03 [10], were developed from genomic libraries of pummelo in California. These markers were preliminarily screened with 9 genotypes (Table 1). Only polymorphic markers were used with the samples.

PCR Reaction and Electrophoresis

The total volume of the PCR product was 25 mL, which contained genomic DNA (50 ng/μL), 1X PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 μM forward and reverse primers, and 0.04 U Taq DNA polymerase (Fermantas, EU). Cycling conditions were 94^{o}C for 3 min., followed by 35 cycles of 94^{o}C for 1 min., 45-65^{o}C for 1 min. (annealing temperature being specific for each marker), 72^{o}C for 2 min. and a final extension of 72^{o}C for 5 min. PCR reactions were performed in a T1 Thermocycler machine (Biometra, Germany).

Five microliters of PCR products mixed with 5 μL sequencing dye (98% formamide, 0.025% bromphenol blue, 0.025% xylenecyanol, 10mM EDTA) were size-separated in 16.5×22 cm^2 denaturing 6% polyacrylamide gel electrophoresis (3 hr and 293 V) and compared with 1kb ladder DNA (SibEnzyme, Russia). The gel was visualised by silver staining [21]. The PhotoCaptMw program (ETS Vilber-Lourmat, France) was used to estimate band sizes from gel images.

Maejo Int. J. Sci. Technol. **2016**, *10*(02), 209-219

Table 1. Pummelo cultivars and grapefruit used in this study

Number	Cultivar name	Source	Use	Code	Pedigree
1	Kao Nam Phueng [z]	C	Commercial	K	-
2	Kao Pan [z]	C	Commercial	KPA	-
3	Kao Pong [z]	C	Commercial	KPU	-
4	Kao Pan seedless [z]	C	Commercial	KS	-
5	Kao Yai [z]	C	Commercial	KY	-
6	Sri Non [z]	C	Local	S	-
7	Bang Kun Non	C	Commercial	BN	-
8	Kao Khom	C	Commercial	KH	-
9	Tong Dee [z]	C	Commercial	T	-
10	Dwarf Tong Dee	C	Commercial	TD	-
11	Tong Dee Nakhon Sri [z]	C	Commercial	TN	-
12	Thap Tim	C	Local	TT	-
13	Ta Chai 30	N	Selection	TC 30	Offspring of Tong Dee
14	Ta Chai 32	N	Selection	TC 32	Offspring of Tong Dee
15	Ta Chai 72	N	Selection	TC 72	Offspring of Tong Dee
16	Ta Chai 73	N	Selection	TC 73	Offspring of Tong Dee
17	Ta Chai 90	N	Selection	TC 90	Offspring of Tong Dee
18	Ta Chai 109	N	Selection	TC 109	Offspring of Tong Dee
19	Ta Chai 130	N	Selection	TC 130	Offspring of Tong Dee
20	Ta Chai 136	N	Selection	TC 136	Offspring of Tong Dee
21	Ta Chai 180	N	Selection	TC 180	Offspring of Tong Dee
22	Ta Khoy	N	Commercial	TA	-
23	Ta Khoy Khoey 7	N	Local	TA 7	-
24	Ta Khoy Sa Thong Kham	N	Local	TAST	-
25	Ta Khoy Som Khit	N	Local	TAS	-
26	Ta Khoy Phrom Phi Ram	N	Local	TAP	-
27	Ta Khoy Wat Ka Hnun	N	Local	TAW	-
28	Ta Khoy Sa Ngat Uncle	N	Local	TAU	-
29	TK4	N	Local	TK4	-
30	Som Phon	N	Local	SP	-
31	Red Som Phon	N	Local	SPR	-
32	Kao Taeng Kwa	C	Commercial	KT	-
33	Ma Tum 2S32	N	Local	M	Offspring of Ta Khoy
34	Khiaw Ma Naw	N	Local	GR	-
35	Mano Rom	N	Local	MN	-
36	Som Krun	N	Commercial	SK	-
37	Ta Phua	N	Local	TP	-
38	Phu Rue 5	N	Local	P 5	-
39	Chaiya Phum 1	N	Local	CP 1	-
40	Sri Vara	N	Local	SV	-
41	Number 3	N	Local	N 3	-
42	Number 6	N	Local	N 6	-
43	Chandler	USA	Foreign[3]	C	-
44	African shaddock	USA	Foreign	AS	-
45	Red Shaddock	USA	Foreign	RS	-
46	Aro Vatee	S	Local	AV	-
47	Khom Had Yai	S	Local	HH	-
48	Chao Sa Woey	S	Local	JS	-
49	Pu Go	S	Local	PG	-
50	Ro Tee	S	Local	RO	-
51	Phata Lung	S	Local	PT	-

Maejo Int. J. Sci. Technol. **2016**, *10*(02), 209-219

Table 1. (continued)

Number	Cultivar name	Source	Use	Code	Pedigree
52	Phata Via	S	Commercial	PV	-
53	Thap Tim Siam	S	Local	TTS	-
54	Grapefruit	USA	Germplasm	G	-

Note: C = pummelo cultivar grown in central Thailand, N = pummelo cultivar grown in northern Thailand, S = pummelo cultivar grown in southern Thailand, USA = pummelo cultivar from USA, Z = pummelo used for primer screening

Data Analysis

The SSR profiles were scored as binary data and the original matrix was used to calculate the allele frequencies for each locus. The assumption of allele frequencies was based on the Hardy–Weinberg equilibrium and was calculated with 2n = 18 for each pummelo cultivar. Then expected He or genetic diversity for a genetic marker was calculated from the sum of the squares of allele frequencies [22] using the formula: $He = 1 - \sum_{i=0}^{k} P_i^2$, where P_i^2 is the genotypic frequency of the A_iA_i genotype.

The polymorphism information content (PIC) value was calculated using the method described by Botstein et al. [23] and the formula: $PIC = 1 - \sum_{i=0}^{k} P_i^2 - 2\sum_{i=1}^{k}\sum_{j=i+1}^{k} P_i^2\, P_j^2$, where P_i^2 is the genotypic frequency of the A_iA_i genotype and P_j^2 is the genotypic frequency of the A_jA_j genotype.

The probability of identity (PI) value of two individuals chosen at random in a population was calculated by the method described by Kaul et al. [24] using the formula: $PI = \sum_{i=1}^{k}(P_i^2)^2 + \sum_{i=1}^{k}\sum_{j=i+1}^{k}(2P_i P_j)^2$, where P_i^2 is the genotypic frequency of the A_iA_i genotype and $2P_iP_j$ is the genotypic frequency of the A_iA_j genotype.

The combined PI of two individuals belonging to more than two SSR markers was calculated using the formula: $PI_{combined} = \prod_{n=1}^{m} PI = (PI_1)(PI_2)\ldots(PI_m)$, where m is the number of SSR markers.

The similarity index (SI) was calculated using the NTSYS software PC version 2.00 [25], and a similarity index matrix was obtained. The cluster analysis was based on similarity matrices using the unweighted pair-group arithmetic average (UPGMA) method, and the relationships between pummelo cultivars were visualised using dendrograms. The co-phenetic correlation coefficient was calculated for the dendrograms after the construction of a co-phenetic matrix. Afterwards, the genetic diversity between pummelo cultivars was visualised in the two-dimensional form of the principal coordinate analysis (PCoA) method using NTSYS software PC version 2.00 [25].

RESULTS AND DISCUSSION

Thirteen SSR primers ran successfully with nine sampled genotypes. Ten primers produced polymorphic markers (Figure 1 and Table 2) while three primers were monomorphic. The monomorphic markers were CAC23, CT02 and CT21; the latter two were developed from pummelo genomic libraries. For the sequence data of pummelo, only 441 nucleotide and 58 EST sequences have been deposited in the GENBANK as of March 2016. At present, sufficient sequence data for

Maejo Int. J. Sci. Technol. **2016**, *10*(02), 209-219

Thai pummelos is unavailable. In future, SSR markers developed from genomic libraries of Thai pummelos will need to be synthesised if these relationships are to be examined further.

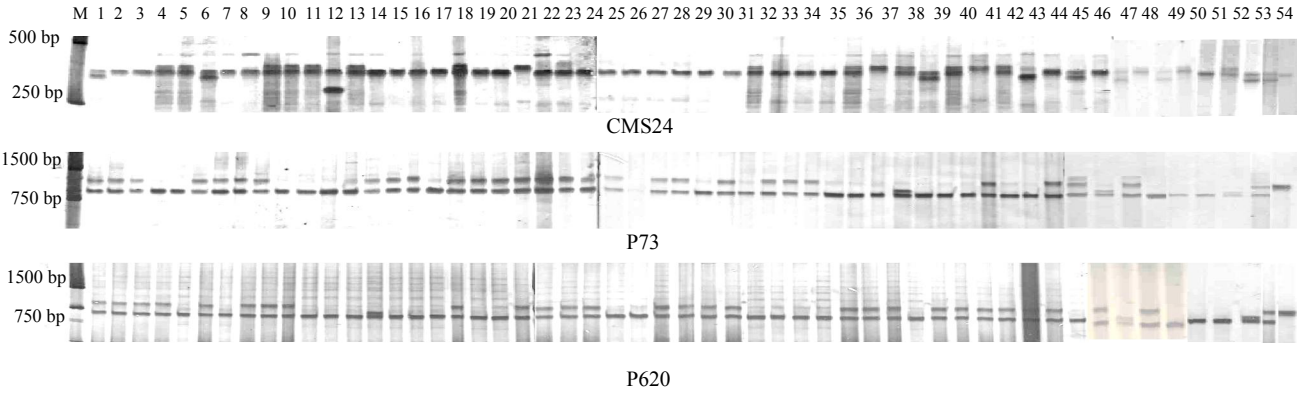

Figure 1. Electrophoretic patterns of 53 pummelo cultivars and grapefruit with SSR marker CMS24, P73 and P620 (M = 1kb ladder DNA; numbers 1-53 = pummelo cultivars as in Table 1; 54 = grapefruit)

Table 2. SSR markers, allelic loci, size range, values of expected heterozygosity (He), polymorphic information content (PIC), probability of identity (PI) and combined PI of 10 SSR markers

SSR marker	Size range (bp)	No. of alleles	He	PIC	PI	1 in ... (clone)	PI combined	1 in ... (clone)
AG14	180-230	4.00	0.66	0.61	0.16	6	0.16	6
P1826	587-1108	3.00	0.63	0.55	0.22	5	0.04	28
GT03	220-237	3.00	0.59	0.50	0.27	4	9.50×10^{-3}	105
CT19	190-213	3.00	0.51	0.44	0.31	3	2.95×10^{-3}	339
AC01	223-243	4.00	0.57	0.48	0.28	4	8.25×10^{-4}	1,212
CMS24	290-377	4.00	0.44	0.40	0.36	3	2.97×10^{-4}	3,367
TAA41	328-399	3.00	0.40	0.36	0.41	2	$1.22 \times 10\text{-}^{4}$	8,213
CMS4	373-443	3.00	0.50	0.40	0.35	3	4.26×10^{-5}	23,465
P73	550-750	3.00	0.43	0.36	0.40	3	1.70×10^{-5}	58,662
P620	1000-1200	3.00	0.44	0.36	0.40	3	6.82×10^{-6}	146,656
Mean		3.30	0.52	0.45				

The 10 polymorphic primers were later used with 53 pummelos and a grapefruit. Ten SSR primers generated a total of 33 markers (180-1200 bp) with the highest number of 4 alleles and the lowest number of 3 alleles per locus. The value of He ranged from 0.40 (TAA 41) to 0.66 (AG14), with an average of 0.52 per locus (Table 2). Barkley et al. [10] presented an average He value of 0.42 in 89 pummelo accessions and hybrids using 24 SSR markers, which indicated a moderate level of polymorphism. Yong et al. [17] found an average He value of 0.25 for 110 pummelo cultivars using AFLP and SSR markers. Thus, the material examined here has greater genetic diversity of pummelo than that examined in California or China.

Comparing information of the He level in pummelo to that in other cross-pollinated plants, Wunsch and Hormaza [26] reported an average He value of 0.49 in 72 sweet cherry genotypes using SSR markers and proposed a moderate level of polymorphism. In 19 *Dendrobium* orchid cultivars

Maejo Int. J. Sci. Technol. **2016**, *10*(02), 209-219

and 24 accessions, Yoocha [27] estimated an average He value of 0.74, indicating a high level of polymorphism. In the present study, the He value of 0.52 can be rated as a moderate level of polymorphism. Pummelo is self-incompatible and the seed is monoembryonic. As a result, the He of Thai pummelo could have been higher. The unexpected lower level is probably due to long-term cultivation with an asexual propagation method and no systematic germplasm management. The moderate value of He in Thai pummelo should be a warning sign that further narrowing of the genetic base could take place unless proper germplasm conservation and breeding efforts are initiated.

The allelic PIC values ranged from 0.36 (P73, P620 and TAA41) to 0.61 (AG14), with an average of 0.45 per marker. The PIC value is commonly used to measure polymorphism for a marker locus [28]. An average of 0.45 is considered as a moderate level of polymorphism when using 10 SSR markers. If SSR markers were developed from the genomic libraries of Thai pummelos, the PIC value might be higher than 0.45. The PI value expresses the likelihood of finding two individuals with the same genotype for a certain locus in the population [29]. The values of PI range from 0.16 (AG14) to 0.41 (TAA41). The combined PI value of the 10 SSR markers was 6.82×10^{-6} or 1 in 1.46×10^{5} (Table 2), indicating the chance of finding two individuals with the same genotype in the pummelo population. Therefore, these 10 SSR markers can be very useful for pummelo identification.

Pummelo samples in the present study were 50 Thai pummelo cultivars from northern, central and southern Thailand, 3 pummelo cultivars from the US and 1 grapefruit. The fifty cultivars of pummelo represent a wide range of types of Thai pummelos. These samples present a variation in pulp colour (white to red) and fruit shape (oblate, spheroid and pyriform) [2, 30]. The SI values of the 53 pummelo cultivars and one grapefruit range from 0.44 to 1.0. The clustered genetic relationship was examined using the UPGMA method. These pummelos can be clustered into seven groups and one out-group at SI = 0.53 (Figure 2). The co-phenetic correlation coefficient (r) is a powerful value for examining the goodness of fit of the clustering analysis and is high (r = 0.84) in this study. Therefore, the dendrogram is considered a good representation of the genetic similarity among samples [24]. Seventy-two sweet cherry genotypes have been characterised with 27 SSR markers and the relationships visualised in a dendrogram with the highest co-phenetic correlation coefficient being 0.66 [26].

In the dendrogram Group 2 has the highest number (20) of pummelo cultivars, consisting of commercial cultivars (KPU, TA and T), hybrids of 'Tong Dee' cultivar (TC32, TC72, TC73, TC90, TC130 and TC136) and a superior genotype of 'Ta Khoy' cultivar (M, TA7, TAST, TAP, TAW, TAU and TK4). Some hybrids of 'Tong Dee' (TC30, TC109 and TC180) are classified into Group 3. The difference among these hybrids represents the genetic variability of the pummelo's natural hybridisation because pummelo is self-incompatible [3, 4]. However, they can be placed in the same group when the dendrogram is classified at SI value = 0.55. All commercial pummelo cultivars are clustered in Groups 1-3. Pummelos in Groups 6-7, consisting of foreign cultivars (C and RS), local cultivars (PG, RO, CP1, PV and HH) and the grapefruit, are more diverse than the commercial cultivars (SI = 0.44). Nevertheless, more foreign and local cultivars should be analysed to verify the current information. This study shows that cultivars 'Kao Pan Seedless' (KS), 'Kao Yai' (KY), 'Kao Taeng Kwa' (KT), 'Sri Vara' (SV), 'Phata Lung' (PT) and N6 have identical SSR genotypes with SI = 1.00. 'Kao Yai' and 'Kao Taeng Kwa', which were studied by Nartvaranant and Nartvaranant [6] and reported to have no differences using AFLP technique, are also found to be identical in this study. Thus, it can be concluded that they are of the same genotype.

Maejo Int. J. Sci. Technol. **2016**, *10*(02), 209-219

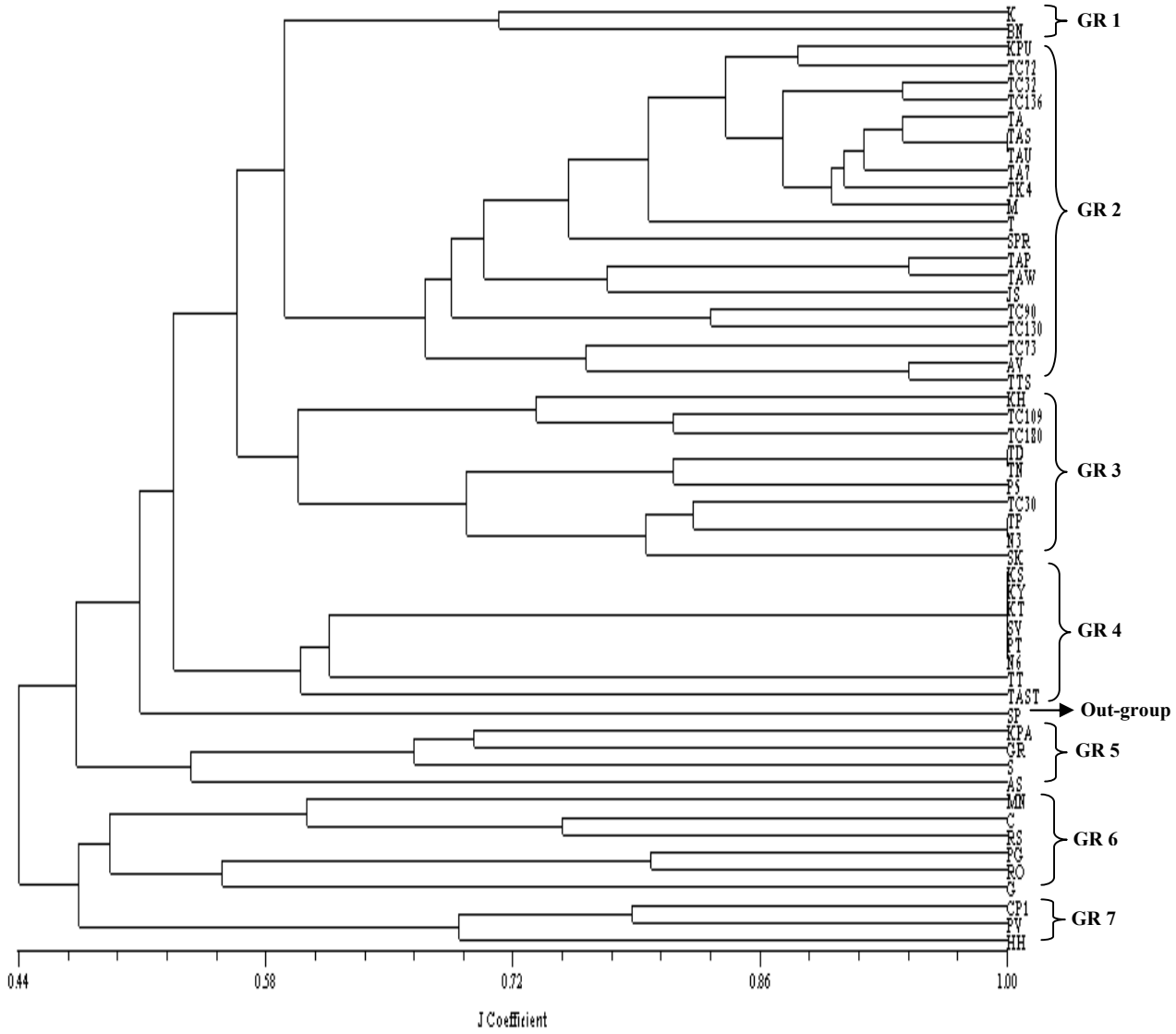

Figure 2. UPGMA dendrogram of 53 pummelo cultivars and a grapefruit using 10 SSR markers and co-phenetic correlation value (r) = 0.84

The grapefruit is classified into Group 6, although the species (*C. paradisi*) is probably a derived hybridisation between pummelo and sweet orange [31]. Corazza-Nunes et al. [11] indicated that grapefruit is associated very closely with pummelo with an average SI value of 0.80. In this study the grapefruit sample has an average SI value of 0.44 when compared with Thai commercial pummelos.

One out-group is 'Som Phon' (SI = 0.53), which was collected from Phichit province in northern Thailand. This is a local cultivar that is moderately tolerant to root rot and *Phytophthora* foot rot. The fruit is of pyriform shape and a light green to yellow green when it ripens with white flesh and a fresh weight of 0.8-1.2 kg [32]. The majority of pummelo cultivars from northern Thailand are presented in Groups 2-4. However, 'Som Phon' presents genetic information related to these groups only when the dendrogram is classified at SI value = 0.50.

The PCoA reveals a comparable grouping structure with the cluster analysis (Figure 3). The PCoA calculates a distance matrix and produces a graphical configuration in a low-dimensional (typically two or three) Euclidean space [33]. It can reduce the complexity of data and corresponds

Maejo Int. J. Sci. Technol. **2016**, *10*(02), 209-219

to a dendrogram. The genetic relationship of pummelos as grouped by PCoA is consistent with the UPGMA dendrogram; similar cultivars are arranged in the same group. The positions of many cultivars (SI = 1.00) are presented on the same level (identical genotype), for example 'Kao Pan seedless' (KS), 'Kao Yai' (KY), 'Kao Taeng Kwa' (KT), 'Sri Vara' (SV), 'Phata Lung' (PT) and N6 (Figure 3).

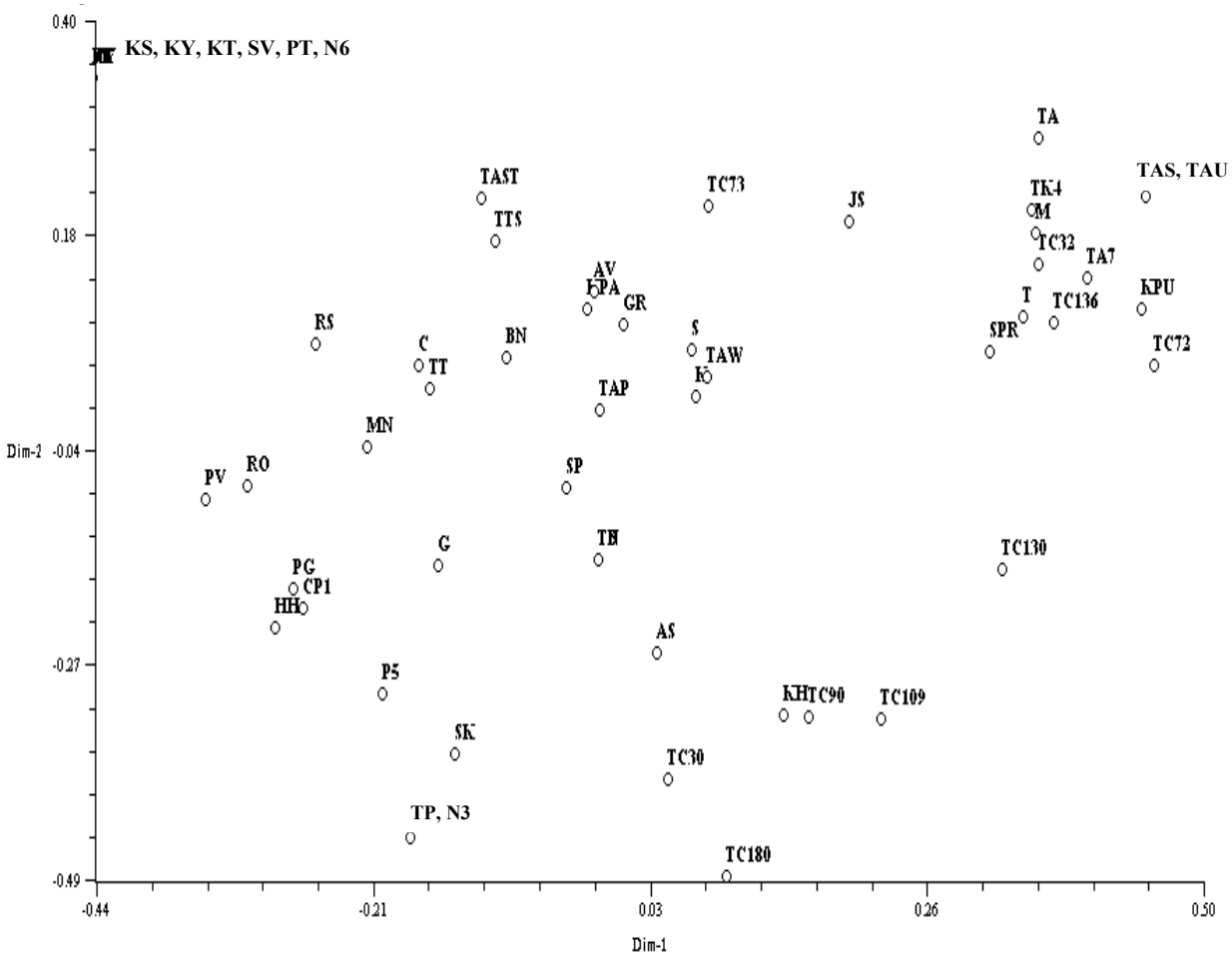

Figure 3. Genetic relationships among 53 pummelo cultivars and one grapefruit by 10 SSR markers and presented using two-dimensional principal coordinate analysis

CONCLUSIONS

Commercial and local cultivars of Thai pummelos show a moderate level of polymorphism as evaluated by 10 SSR markers. The PI value of these markers is high, indicating their usefulness in genetic identification. The 53 cultivars and one grapefruit can be clustered into seven groups. Group 2 with 20 cultivars (SI = 0.53) consists of commercial cultivars and hybrids from 'Thong Dee' and 'Ta Khoy'. Some commercial cultivars show identical SSR genotypes but have been given different names. Local and foreign cultivars are more diverse than the commercial cultivars (SI = 0.44).

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Maejo Int. J. Sci. Technol. **2016**, *10*(02), 209-219

REFERENCES

1. J. W. Purseglove, "Tropical Crops: Dicotyledons", John Wiley and Sons, New York, **1968**, pp. 504-505.
2. R. Sethpakdee, "Citrus Production in Thailand", Department of Horticulture, Kasetsart University, Kamphaengsaen Campus, Nakhon Pathom, **2002**, pp. 24-25 (in Thai).
3. R. K. Soost, "Incompatibility alleles in the genus *Citrus*", *Proc. Amer. Soc. Hort. Sci*., **1965**, *87*, 176-180.
4. R. K. Soost, "The incompatibility gene system in citrus", Proceedings of 1st International Citrus Symposium, **1969**, Riverside (CA), USA, pp.189-190.
5. Office of Agricultural Economics, "Agricultural economic information", **2014**, http://www.oae.go.th/download/download_journal/commodity55.pdf (Accessed: October 2015) (in Thai).
6. P. Nartvaranant and K. Nartvaranant, "Analysis based on AFLP markers of the genetic variations and their relationships for pummelo cultivars grown in the central region of Thailand", *Songklanakarin J. Sci. Technol*., **2011**, *33*, 499-508.
7. S. Kongsri and U. Boonprakob, "Identification of ambiguous pummelos using simple sequence repeat (SSR) marker", *Agric. Sci. J*., **2006**, *37(Suppl.)*, 589-592 (in Thai).
8. B. Wu, G. Zhong, J. Yue, R. Yang, C. Li, Y. Li, Y. Zhong, X. Wang, B. Jiang, J. Zeng, L. Zhang, S. Yan, X. Bei and D. Zhou, "Identification of pummelo cultivars by using a panel of 25 selected SNPs and 12 DNA segments", *PLOS ONE*, **2014**, *9,* e94506.
9. R. Ahmad, D. Struss and S. M. Southwick, "Development and characterization of microsatellite markers in *Citrus*", *J. Amer. Soc. Hort. Sci.*, **2003**, *128*, 584-590.
10. N. A. Barkley, M. L. Roose, R. R. Krueger and C. T. Federici, "Assessing genetic diversity and population structure in a citrus germplasm collection utilizing simple sequence repeat markers (SSRs)", *Theor. Appl. Genet*., **2006**, *112*, 1519-1531.
11. M. J. Corazza-Nunes, M. A. Machado, W. M. C. Nunes, M. Cristofani and M. L. P. N. Targon, "Assessment of genetic variability in grapefruits (*Citrus paradisi* Macf.) and pummelos (*C. maxima* (Burm.) Merr.) using RAPD and SSR markers", *Euphytica*, **2002**, *126*, 169-176.
12. D. Q. Fang and M. L. Roose, "Identification of closely related citrus cultivars with inter-simple sequence repeat markers", *Theor. Appl. Genet*., **1997**, *95*, 408-417.
13. M. J. Hayden and P. J. Sharp, "Targeted development of informative microsatellite (SSR) markers", *Nucl. Acids Res*., **2001**, *29*, e44.
14. W. Powell, G. C. Machray and J. Provan, "Polymorphism revealed by simple sequence repeats", *Trends Plant Sci.*, **1996**, *1*, 215-222.
15. M. L. Roose, C. T. Federici and G. P. Copenhaver, "Genetic diversity in pummel (*Citrus maxima* (Burm.) Merrill), citron (*C. medica* L.), and trifoliate orange (*Poncitrus trifoliate* (L.) Raf) evaluated using RFLPs", *Hort. Sci*., **1992**, *27*, 624.
16. J. M. H. Kijas, M. R. Thomas, J. C. S. Fowler and M. L. Roose, "Integration of trinucleotide microsatellites into a linkage map of Citrus", *Theor. Appl. Genet*., **1997**, *94*, 701-706.
17. L. Yong, S. Zhong-hai, L. De-chun, W. Bo and T. Jian-jun, "Assessment of the genetic diversity of pummelo germplasms using AFLP and SSR markers", *China Agric. Sci.*, **2005**, *38*, 2308-2315 (in Chinese).
18. M. Liang, X. Yang, H. Li, S. Su, H. Yi, L. Chai and X. Deng, "De novo transcriptome assembly of pummelo and molecular marker development", *PLOS ONE*, **2015**, *10,* e0120615.

Maejo Int. J. Sci. Technol. **2016**, *10*(02), 209-219

19. L. Chai, M. K. Biswas, H. Yi, W. Guo and X. Deng, "Transferability, polymorphism and effectiveness for genetic mapping of the pummelo (*Citrus grandis* Osbeck) EST-SSR markers", *Sci. Hort.,* **2013**, *155*, 85-91.
20. G. K. Agrawal**,** R. N. Pandey and V. P. Agrawal, "Isolation of DNA from *Choerospondias axillaris* leaves", *Biotechnol. Biodiv. Lett*., **1992**, *2*, 19-24.
21. B. J. Bassam and G. Caetano-Anolles, "Silver staining of DNA in polyacrylamide gels", *Appl. Biochem. Biotechnol.*, **1993**, *42*, 181-188.
22. M. Nei, "Estimation of average heterozygosity and genetic distance from a small number of individuals", *Genetics*, **1978**, *89*, 583-590.
23. D. Botstein, R. L. White, M. Skolnick and R. W. Davis, "Construction of a genetic linkage map in man using restriction fragment length polymorphisms", *Am. J. Hum. Genet*., **1980**, *32,* 314-331.
24. R. Kaul, A. Singh, R. K. Vijh, M. S. Tantia and R. Behl, "Evaluation of the genetic variability of 13 microsatellite markers in native Indian pigs", *J. Genet*., **2001**, *80,* 149-153.
25. F. J. Rohlf, "NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System Version 2.00; User Guide", Applied Biostatistics Inc., New York, **1997**.
26. A. Wunsch and J. I. Hormaza, "Molecular characterisation of sweet cherry (*Prunus avium* L.) genotypes using peach [*Prunus persica* (L.) Batsch] SSR sequences", *Heredity*, **2002**, *89*, 56-63.
27. T. Yoocha, "Microsatellite DNA markers for dendrobium orchid cultivars and pedigree identification", *MS Thesis*, **2006**, Kasetsart University, Thailand.
28. S. Shete, H. Tiwari and R. C. Elston, "On estimating the heterozygosity and polymorphism information content value", *Theor. Popul. Biol.*, **2000**, *57*, 265-271.
29. M. Kirst, C. M. Cordeiro, G. D. Rezende and D. Grattapaglia, "Power of microsatellite markers for fingerprinting and parentage analysis in *Eucalyptus grandis* breeding populations", *J. Hered*., **2005**, *96*, 161-166.
30. P. Thayamanon, "Characterization of Pummelo Cultivars", Horticulture Research Institute, Department of Agriculture, Bangkok, **1998** (in Thai).
31. R. W. Scora, J. Kumamoto, R. K. Soost and E. M. Nauer, "Contribution to the origin of the grapefruit, *Citrus paradisi* (Rutaceae)", *Syst. Bot*., **1982**, *7*, 170-177.
32. A. Zuur, E. N. Leno and G. M. Smith, "Analyzing Ecological Data", Springer-Verlag, New York, **2007**, pp.259-261.
33. Phichit Agricultural Research and Development Center, "Study on pummelo rootstock tolerance to phytophthora foot rot", **2010**, http://www.svppijit.com/Abstract09.html (Accessed: October 2015) (in Thai).