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Application of Microsatellite Markers for Identification of Wine Grape Varieties in Thailand

Alongkorn Sripholtaen[1], Charoen Charoenchai[1] and Hathairat Urairong[2]*

[1]Division of Crop Production Technology, Faculty of Agricultral Technology
Rajamangala University of Technology Thanyburi
[2]Biotechnology Research and Development Office, Department of Agriculture,
Ministry of Agriculture and Cooperatives
[1]Corresponding author: c_charoen@yahoo.com*

Abstract

The use of Microsatellite markers for identifying grapevine varieties. Eighteen primer pairs were selected for identifying amplify 29 grapevine varieties. The result shower that a total of 133 alleles with an average of 7.4 alleles per locus were found to be polymorphic. All alleles size were approximately 112-262 base pair. The cluster analysis using unweighted pair group method with arithmetic mean (UPGMA) based on genetic dissimilarity matrix with 0.9. Twenty nine grapevine varieties were clustered into two major groups. This research indicated that the microsatellite markers could be used to examine and compare the genetic resources among closely samples. The microsatellite maker is useful for discrimination and analysis of genetic diversity of grapevine varieties, and also for grapevine breeding in the future.

Keywords : *Grapevine, DNA fingerprint, Microsatellite marker*

1. Introduction

Grapevine (Vitis vinifera L.) is one of the most important crop plants of the world. The crop has a wide adaptability, and grapes can be grown under temperate, sub-tropical and tropical climate conditions were found that grape can be adopted and grown well country of the Thailand. The main varieties of table grapes are white Malaga, Beauty Seedless and Cardinal. These three varieties are mainly used for table grapes as well as for wine making. The major varieties of wine grapes are Shiraz and Chenin Blanc. The grape industry in Thailand has been a success since this time. The many varieties (table, wine and raisin) for field evaluation under local conditions were considered an important objective for the advancement of grape industries throughout the region, in producing fresh and processed grape products of a quality (9).

Today, some grape varieties have similar morphological characteristics causing difficult to identify the certain traits,

thus synonyms and homonyms. It means that there are a lot of different varieties mentioned under the same name (homonyms), and there are varieties, what has more than one name (synonyms). Traditionally, varieties characterization relied on a plant morphological description method is easy (6). However, these observations are time consuming and error-prone due to environmental variations that may alter the expression of the measured characteristics.

The developments of microsatellite markers in DNA analysis for the discrimination of varieties through the application of the microsatellite (SSR) fingerprinting in viticulture have become the technique of choice for varieties identify and distinction (3,10). Microsatellite are the best markers to discriminate the varieties. In fact, This et al. (11) demonstrated the usefulness of a standard set of microsatellite for the identification of grape varieties. The present study intended to complement detailed ampelographic characterization of grapevine germplasm resources by using microsatellite markers useful for identification and analysis of genetic diversity of grapevine varieties, and also for grapevine breeding in the future.

2. Materials and Methods

2.1 Plant materials

The leaf samples of 29 grapevine varieties; Shiraz, Cabernet Sauvignon, Chenin Blanc, Colombard, Tempranillo were collected in PB Valley Khao Yai Winery, Nakhon Ratchasima province; Chardonay, Riesling, Guetramimer, Zintandel, Niagara, Carbernet Franc, Rubired, Babera, Macabau Blanc, Faustino, Delaware, Merlot, Pinut Noir and Marechal Foch were collected Suranaree University of Technology, Nakhon Ratchasima province; Shiraz, Shiraz 174, Cabernet Sauvignon, Chenin Blanc 220, Chenin Blanc 880, Colombard 607B, Colombard 607C, Colombard 625, Tempranillo 776, Tempranillo RJ51, were collected from Hua Hin Hills Vineyard, Prachuap Khiri Khan province. Fresh foliage samples were frozen and kept at -20 ^{o}C for genomic DNA extraction.

2.2 Genomic DNA extraction

The genomic DNA was extracted from leaf samples using CTAB method which modified (1). Briefly, 0.1g of leaves tissues were grounded in liquid nitrogen into a fine powder and transferred into an Eppendorf tube with 700 ml of 2X CTAB [2% CTAB, 0.5M NaCl, 1M Tris-HCl; pH 8.0, 0.5M EDTA; pH 8.0, 2%w/v (CTAB)] and 2-mercaptoethanol (2%) mixing well and incubated at 60^{o}C for 30 min, then the chloroform:isoamyl alcohol (24:1) 700 µl was added and mixed thoroughly. The sample has been collected by centrifuging at 12,000 rpm for 10 min. To the upper phase collected, 0.6 volumes of isopropanol and 0.1 volumes of 3M sodium acetate (pH 5.2) were added and thoroughly mixed. Tubes were kept at -20 ◦C for 30 min. The precipitate was collected by centrifuge at 12,500 rpm for 10 min at 4◦C and washing with 75% ethanol. The remaining ethanol was evaporated at room temperature and the pellet dissolved in 100 µl of TE (2M Tris-HCl, 0.5M EDTA; pH 8.0). The integrity of extraction DNA (purity and concentration) was evaluated using spectrophotometer at 260 and 280 nm and necessary dilutions were done, followed by verification with 1% agarose gel

electrophoresis. The DNA samples were stored at -20°C until further analysis.

2.3 Microsatellite marker Selection

All 35 microsatellite primer assayed using the DNA samples of random selected five grapevine varieties (Shiraz, Cabernet Souvignon, Chenin Blanc, Tempranillo and Colombard). The PCR reaction was performed using 20 µl of PCR reaction mixture containing 2 µl of 10X PCR buffer $(NH_4)_2SO_4$), 0.8µl of MgCl2 (50mM), 2µl of dNTPs (100mM), 1µl of primer (10mM), 0.2 µl of Tag DNA polymerase (5 Unit/ml of Fermentas) and 40ng of genomic DNA. The PCR were carried out in Gene Amp 9700 with the following profile; 95°C for 7 min followed by 40 cycles of 95°C for 40s, 55°C for 40s, and 72°C for 40s, and a final extension at 72°C for 7 min. Fragment differentiation and allele size determination were separated by automatic electrophoresis with ABI Prism™ 377 DNA Sequences were analyzed by Genescan software using DNA size standard GS-500 (ROX). Differentiation of microsatellite primer pairs was displaied by Genotyper software (2). The tested primers were excel uoled from the study if monomorphic or poor amplified products were found.

2.4 Microsatellite marker analysis

All selected microsatellite primer were individually amplified using DNA of each 29 grapevine varieties. The PCR product were electrophoresed on ABI Prism™ 377 DNA Sequences. The DNA fragments were analyzed and sized to assigned the specific alleles by GeneScan and Genotyper software. Cluster analysis using the unweighted pair group method with arithmetic (UPGMA).

3. Results and Discussion

3.1 Genomic DNA extraction

The effective of DNA extraction from 29 grapevine varieties were obtained by spectrophotometer at 260 and 280 NM as between 1.85 to 2.00, which DNA of highly pure.

3.2 Microsatellite marker Selection

To select microsatellite markers The thirty primer pairs were amplify the with five random selected grapevine varieties. The result showed that final of 18 microsatellite primer pairs as FAM02, FAM32, FAM44, FAM46, FAM57, FAM59, FAM60, FAM75, FAM79, FAM126, FAM129, FAM138, VVS2, VVMD5, VVMD7, VVMD27, VrZAG62 and VrZAG79 (Table 1). This result, similar to Huang et al. (8), demonstrated that microsatellite loci FAM02, FAM32, FAM44, FAM57 and FAM60 were used for identification of grapevine varieties in Riesling, Cabernet, Summit, Noble. The functionalities of markers are a useful for grape genotyping and genome mapping. (3, 4, 10, 12). Moreover, the three loci; VVS2, VVMD5 and VVMD7 were used for identification of sixty-six grapevine and rootstock cultivars genotype from an Austrian germplasm collection. This study indicated that microsatellite markers useful for identification and analysis of genetic diversity of 29 grapevine varieties, the results were shown in table 4.

3.3 Microsatellite marker analysis

Eighteen microsatellite marker loci were selected to use for identifying the DNA fingerprinting in 29 grapevine varieties.The result showed that total of 133 DNA polymorphic detected from all grapevine and the alleles size were

approximately 112-262 bp. The polymorphic band patterns of all primers pair range of 3-12 alleles and an average of alleles were 7.4 alleles per locus (Table 2). The highest polymorphic alleles number were 12 alleles at the locus of FAM02, the size of each alleles started from 188, 190,198, 200, 204, 208, 216, 218, 220, 226, 232 and 234 base pair.The lower level of polymorphic alleles number, 11 and 10 alleles were found in locus VrZAG79, FAM126 and VVMD27, FAM79 respectively. Three alleles were the lowest polymorphic alleles found in primer FAM44, FAM46 and FAM129 (Table 2). The DNA fingerprint data of 29 grapevine varieties using 18 microsatellite primer , the total of 522 data were obtained and summarized in table 3. For example, three are 11 alleles present at locus VrZAG79. However, alleles size 230 bp was found only in Zintandel and Niagara varieties. Moreover, the alleles size 232 and 244 bp can be used as unique breeding for identifying Riesling and Shiraz varieties, respectively. The data reported here (Table 3) clearly supports the 18 selected microsatellite primer suitable to be use for identification 29 grapevine varieties.

The cluster analysis using unweighted pair group method with arithmetic mean (UPGMA) based on genetic similarity distance shown in Table 4.

The results showed that 29 grapevine varieties were clustered into two major groups; A and B. The genetic distance between two groups are very genetically differentiated more than 0.9 (Figure 1). The two major groups of grapevine varieties were separated to red and white grapes (A, B). The dendrogram of genetic relationships of grapevine varieties divided into four groups as a1, a2, b1 and b2. Among the studied varieties, dissimilarity based on proportion of shared alleles ranged from zero to one. The other varieties appear as groups of identical individuals and closely related varieties in the dendrogram (Figure 1).

Groups a1 consist with 3 samples of a red grape which Tempranillo RJ51 and Tempranillo 776 were collected from Hua Hin Hills Vineyard, Prachuap Khiri Khan province and Tempranillo was collected from PB Valley Khao Yai Winery, Nakhon Ratchasima province. The results showed that the three varieties of red grape have no genetic differentiation by using 18 primer pairs of microsatellite marker with genetic distance as 0.00 (Table 4), they were thought to be members of the same group. It is possible that all of three varieties are the same species, while the genetic relationships of thousand grape varieties in group a1 and a2 showed genetic distance average of 0.6 and 0.4, respectively (Figure 1). This results similarity of microsatellite marker analysis for identification of grapevine varieties in grape products which is Tempranillo (6).

Table1. Nucleotide sequence of 35 microsatellite primer used for DNA fingerprinting of grapevine varieties.

Microsatellite primer	Repeat sequence	sequences of primer		reference
		5'→3'forward	5'→3'-reverse	
VVS2	$(GA)_{22}$	F-CAGCCCGTAAATGTATCCATC	R-AAATTCAAAATTCTAATTCAACTGG	Bowers *et al.*, 1996, 1999
VVMD5	$(CT)_3AT(CT)_{11}ATAG(AT)_3$	F-CTAGAGCTACGCCAATCCAA	R-TATACCAAAAATCATATTCCTAAA	Bowers *et al.*, 1996, 1999
VVMD7	$(CT)_{14.5}$	F-AGAGTTGCGGAGAACAGGAT	R-CGAACCTTCACACGCTTGAT	Bowers *et al.*, 1996, 1999
VVMD27	$(CT)_x$	F-GTACCAGATCTGAATACATCCGTAAGT	R-ACGGGTATAGAGCAAACGGTGT	Sefe *et al.*, 1999
VrZAG62	$(GA)_{19}$	F-GGTGAAATGGGCACCGAACACCACGC	R-CCATGTCTCTCCTCAGCTTCTCAGC	Sefe *et al.*, 1999
VrZAG79	$(GA)_{19}$	F-AGATTGTGGAGGAGGGAACCAAACCG	R-TGCCCCCATTTTCAAACTCCCTTCC	Thomas and scott.1993
FAM02	$(TC)_{10}$	F-GCCTTGGACCGAACTATC	R-CTAAGAAACACCATTCATCAG	Huange *et al.*, 2010
FAM04	$(CT)_{10}$	F-GTGACTTACAATCCTTCCAAA	R-AGGGAGAGAGAGAGAGAGAGA	Huange *et al.*, 2010
FAM10	$(AAT)_{13}$	F-TGAAGCACTGATGCTTATTG	R-ACAATGTCACACACAAGGTG	Huange *et al.*, 2010
FAM13	$(CAG)_6$	F-CTCTTCAGGAAACACTGGAG	R-CCTGGAGTTCCTGGTAGATT	Huange *et al.*, 2010
FAM14	$(CTT)_8$	F-AGACCACCATGGATCACTT	R-CTTGATATTCTTAATGGGCG	Huange *et al.*, 2010
FAM18	$(AGA)_7$	F-AGAGAGCAAAGGAACATGAA	R-ACAAACCCTAACCCTAGCTC	Huange *et al.*, 2010
FAM32	$(CAC)_7$	F-AAACTGGACTCCACTGTCTG	R-GTGGAGATGGCACTAATAGC	Huange *et al.*, 2010
FAM35	$(CAG)_7$	F-CACTCTCCAACTCCAGATGT	R-ATGTTTCCCATATTCACAGC	Huange *et al.*, 2010
FAM41	$(AGC)_6$	F-CAGAAGTTGAGAAGTCAGGG	R-ACTTTGGCATTCCTAACTGA	Huange *et al.*, 2010
FAM44	$(AAG)_6$	F-GAGGAGGTGGAAGGAGAA	R-TTTGATAAGGTTGATGGTCC	Huange *et al.*, 2010
FAM46	$(AAAGG)_4$	F-TAACCTCACATCACATCCCT	R-TATTAGGGTCTGCTGCAAAT	Huange *et al.*, 2010
FAM50	$(AG)_{14}$	F-CACAAAGCATGTCCATAAAC	R-GGCTTATGCATTACTGGACT	Huange *et al.*, 2010
FAM57	$(CT)_{16}$	F-CCATCTACCATCACCTTTGT	R-GGAGAAGTGGTATTTGGTGA	Huange *et al.*, 2010
FAM59	$(GCA)_7$	F-GATGGTATACGACGGAGAAA	R-AGAGTACGACCCTTCGATCT	Huange *et al.*, 2010
FAM60	$(CAA)_6$	F-CCTCATCTGGCTTTCATAAC	R-CTGGACAGAACTTGGATCAT	Huange *et al.*, 2010
FAM71	$(AT)_9$	F-AGTCTCTTCAAGTGCCTCAG	R-CTGCATAGACTGACGAAACA	Huange *et al.*, 2010
FAM72	$(CT)_{14}$	F-TCAGTCCAGATTTACCTTGC	R-TCATGTGGTTCTGCAATAGA	Huange *et al.*, 2010
FAM75	$(CTT)_{11}$	F-CCTGTAAACGCTTCAAATCT	R-ATGGCTGAGTCATAGAGAGG	Huange *et al.*, 2010
FAM79	$(AG)_{12}$	F-GCAGAAGCAAGAAGTGAAGT	R-AGATTCAAAGCCACTGAAGA	Huange *et al.*, 2010
FAM81	$(GCC)_8$	F-TTCTCTCAACATACATGGCA	R-GCACTGAATACACTTGGGTT	Huange *et al.*, 2010
FAM102	$(TATG)_5$	F-ACCCATGTTCTCTTCAACAC	R-CGAGAGATTGGAGAGTATCG	Huange *et al.*, 2010
FAM106	$(TCG)_6$	F-TCATCAACATCATCATCCAC	R-GCACTCTTCTCACCTTTGTT	Huange *et al.*, 2010
FAM126	$(AG)_{10}$	F-CGACCTAAGAAACACCATTC	R-CCTTGGACCGAACTATCTG	Huange *et al.*, 2010
FAM129	$(AAAGG)_4$	F-ACATCCCTTTGTTGTCTTCTT	R-ATTTGTGCTGTTGTCTGTTGT	Huange *et al.*, 2010
FAM137	$(TC)_9$	F-CAAACTGTCCAATCCTCATAGT	R-AGTAGAGCCAAGTGTCAAACC	Huange *et al.*, 2010
FAM138	$(GCA)_9$	F-CGAGTGGTAGAGAGGAGAGAG	R-GTTGAGGGTGATGGTAAGG	Huange *et al.*, 2010
FAM144	$(ACC)_9$	F-CACCACTATCACCACTACCAC	R-AGGAGGCGAATGAAGGTC	Huange *et al.*, 2010
FAM145	$(CAA)_7$	F-TCCAACAACAACAACTACTAC	R-AGGAATCTCGTGTCGCTC	Huange *et al.*, 2010
FAM146	$(TC)_9$	F-CAAACTGTCCAATCCTCATAGT	R-AGTAGAGCCAAGTGTCAAACC	Huange *et al.*, 2010

Table 2. The size and number obtained from 29 grapevine samples were amplified with 18 microsatellite primer

Primer	Size of alleles (Base pair)	Number of alleles
VVS2	123, 125, 127, 133, 135, 137, 139, 143, 145 and 151	10
VVMD5	226, 228, 232, 236, 238, 240, 244 and 262	8
VVMD7	235, 237, 243, 247, 249, 251, 255, 257 and 261	9
VVMD27	175, 179, 181, 183,185, 189, 191, 195, 199 and 207	10
VrZAG62	182, 186, 188, 190, 194, 196, 198 and 200	8
VrZAG79	230, 232, 236, 238, 242, 244, 246, 248, 250, 252 and 262	11
FAM02	188, 190, 198, 200, 204, 208, 216, 218, 220, 226, 232 and 234	12
FAM32	191, 194, 197, 203 and 209	5
FAM44	112, 115 and 118	3
FAM46	141, 146 and 151	3
FAM57	132, 134, 136, 138, 140, 142, 154 and 156	8
FAM59	168, 171, 174 and 180	4
FAM60	114, 132, 135, 141 and 144	5
FAM75	142, 145, 148, 151, 157, 160, 163, 166 and 169	9
FAM79	143, 145, 147, 149, 153, 155, 159, 163,165 and 173	10
FAM126	189, 193, 201, 203, 209, 213 219, 221, 223, 227 and 235	11
FAM129	112, 117 and 122	3
FAM138	203, 206, 209 and 212	4
	Total	133
	Average alleles/locus	7.4

Table 3. The size and number of alleles per locus (primer) of DNA fingerprinting obtained from 29 grapevine samples with 18 microsatellite primers. (+ = present, - = absent)

Locus	Alleles size (bp)	Shiraz 174	Shiraz	Shiraz	Cabernet Sauvignon	Cabernet Sauvignon	Chenin Blanc 220	Chenin Blanc 880	Chenin Blanc	Colombard 607B	Colombard 607C	Colombard 625	Colombard	Tempranillo 776	Tempranillo RJ51	Tempranillo	Chardonay	Riesling	Guetramimer	Marechal Foch	Zintandel	Niagara	Carbernet Franc	Rubired	Babera	Macabau Blanc	Faustino	Deraware	Merlot	Pinut Noir
VVS2	123	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
	125	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	+	-	-
	127	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
	133	+	+	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	+	-	-
	135	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
	137	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	+
	139	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
	143	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-
	145	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	+	-	+	-	-	-	-
	151	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	+	+
VVMD5	226	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-	+	+	+	-	+	-
	228	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	+
	232	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
	236	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	+	+	+	-	-	+	-	+	-
	238	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	+	-	-	-	-	+	-	-	-	+
	240	-	-	-	+	+	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	244	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
	262	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
VVMD7	235	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
	237	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	-	-	-	+	-	-	+	+
	243	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	+
	247	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
	249	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	+	-	-	-
	251	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-
	255	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
	257	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
	261	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-
VVMD27	175	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	179	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	+	+	-	-	-	-	-	-
	181	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-
	183	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	185	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	+	-	+
	189	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	+	-	-	-	+	+
	191	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	195	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
	199	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
	207	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
VrZAG62	182	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
	186	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	+	+	+	+	+	+	-	-
	188	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	190	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+
	194	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-
	196	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	+
	198	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-
	200	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	+	-	-

Table 3. (Cont.)

Locus	Alleles size (bp)	Shiraz 174	Shiraz	Shiraz	Cabernet Sauvignon	Cabernet Sauvignon	Chenin Blanc 220	Chenin Blanc 880	Chenin Blanc	Colombard 607B	Colombard 607C	Colombard 625	Colombard	Tempranillo 776	Tempranillo RJ51	Tempranillo	Chardonay	Riesling	Guetramimer	Marechal Foch	Zintandel	Niagara	Carbernet Franc	Rubired	Babera	Macabau Blanc	Faustino	Deraware	Merlot	Pinut Noir
VrZAG79	230	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-
	232	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
	236	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-
	238	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	+	+	-	-	-	-	-	+
	242	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	244	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	246	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	248	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
	250	+	+	+	-	-	+	+	+	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+	-	-
	252	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	+	-
	262	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
FAM02	188	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
	190	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	+	+	-
	198	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-
	200	+	+	+	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+
	204	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-
	208	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
	216	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
	218	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	+	+	-	-	+	-	-	-
	220	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	226	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
	232	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
	234	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
FAM32	191	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	194	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+	-	-	-	-	+	-	-	-
	197	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+
	203	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-	-	-	-	-	-
	209	+	+	+	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
FAM44	112	+	+	+	-	-	+	+	+	+	+	+	+	-	-	-	+	+	+	+	-	+	+	+	-	+	+	+	+	+
	115	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	+	-	+	+	+	+	+	+	-	-
	118	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+	-	-	-	+	+
FAM46	141	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	146	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-
	151	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-
FAM57	132	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
	134	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
	136	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
	138	+	+	+	-	-	-	-	-	+	+	+	+	+	+	+	-	-	+	-	-	-	-	+	+	+	-	-	-	+
	140	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	+	-	-	-	-	-	-
	142	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	-	-	+	-
	154	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	+	+
	156	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-

Table 3. (Cont.)

Locus	Alleles size (bp)	Shiraz 174	Shiraz	Shiraz	Cabernet Sauvignon	Cabernet Sauvignon	Chenin Blanc 220	Chenin Blanc 880	Chenin Blanc	Colombard 607B	Colombard 607C	Colombard 625	Colombard	Tempranillo 776	Tempranillo RJ51	Tempranillo	Chardonay	Riesling	Cuetramimer	Marechal Foch	Zintandel	Niagara	Carbernet Franc	Rubired	Babera	Macabau Blanc	Faustino	Deraware	Merlot	Pinut Noir
FAM59	168	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	+	+	+	+	-	+	-	-	-	-
	171	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
	174	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	-	-	-	+
	180	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
FAM60	114	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	132	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
	135	-	-	-	+	+	-	-	-	+	+	+	+	+	+	+	-	-	-	+	-	+	+	-	-	-	+	+	+	-
	141	+	+	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	+	-	-	-	+
	144	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	-	-	-	+	+	+
FAM75	142	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-
	145	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-
	148	+	+	+	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+	-	+	+	-	+	+	-	+	-	-	-
	151	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
	157	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
	160	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	+	-	-	-	-	-	+	+
	163	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
	166	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-
	169	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	+	+	+	-	-	-	+	-	+	+	-	+	+	+
FAM79	143	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
	145	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-
	147	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-
	149	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	+	-	-	-	-
	153	+	+	+	-	-	-	-	-	+	+	+	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+
	155	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	+	+	+	-	+	-	-	-	-	+	+	-
	159	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
	163	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	+	+	-	-	-	-	+	-
	165	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+
	173	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
FAM126	189	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
	193	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	+	-	-
	201	+	+	+	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
	203	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	+	-	-	+	+
	209	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
	219	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	-	-	-
	213	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
	221	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	+	-	-	-	-	-	-
	223	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	227	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
	235	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-
FAM129	112	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	117	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-
	122	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	+	-	+	+	-	+	-	-	-	-
FAM138	203	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	206	+	+	+	+	+	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-
	209	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+
	212	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 4. The genetic dissimilarity distance of 29 grapevine varieties obtained from analysis using 18 microsatellite primer pairs

Case	Shiraz 174	Shiraz	Shiraz	Cabernet Sauvignon	Cabernet Sauvignon	Chenin Blanc 220	Chenin Blanc 880	Chenin Blanc	Colombard 607B	Colombard 607C	Colombard 625	Colombard	Tempranillo 776	Tempranillo RJ51	Tempranillo	Chardonay	Riesling	Guetramimer	Marechal Foch	Zintandel	Niagara	Carbernet Franc	Rubired	Babera	Macabau Blanc	Faustino	Deraware	Merlot	Pinut Noir
Shiraz 174	0.00																												
Shiraz	0.00	0.00																											
Shiraz	0.00	0.00	0.00																										
Cabernet Sauviqucn	0.57	0.57	0.57	0.00																									
Cabernet Sauviqucn	0.57	0.57	0.57	0.00	0.00																								
Chenin Blanc 220	0.47	0.47	0.47	0.41	0.41	0.00																							
Chenin Blanc 880	0.47	0.47	0.47	0.41	0.41	0.00	0.00																						
Chenin Blanc	0.47	0.47	0.47	0.41	0.41	0.00	0.00	0.00																					
Colombard 607B	0.61	0.61	0.61	0.55	0.55	0.53	0.53	0.53	0.00																				
Colombard 607C	0.61	0.61	0.61	0.55	0.55	0.53	0.53	0.53	0.00	0.00																			
Colombard 625	0.61	0.61	0.61	0.55	0.55	0.53	0.53	0.53	0.00	0.00	0.00																		
Colombard	0.61	0.61	0.61	0.55	0.55	0.53	0.53	0.53	0.00	0.00	0.00	0.00																	
Tempranillo 776	0.55	0.55	0.55	0.76	0.76	0.75	0.75	0.75	0.61	0.61	0.61	0.61	0.00																
Tempranillo RJ51	0.55	0.55	0.55	0.76	0.76	0.75	0.75	0.75	0.61	0.61	0.61	0.61	0.00	0.00															
Tempranillo	0.55	0.55	0.55	0.76	0.76	0.75	0.75	0.75	0.61	0.61	0.61	0.61	0.00	0.00	0.00														
Chardonay	0.61	0.61	0.61	0.75	0.75	0.76	0.76	0.76	0.71	0.71	0.71	0.71	0.73	0.73	0.73	0.00													
Riesling	0.63	0.63	0.63	0.80	0.80	0.67	0.67	0.67	0.73	0.73	0.73	0.73	0.71	0.71	0.71	0.29	0.00												
Guetramimer	0.59	0.59	0.59	0.69	0.69	0.63	0.63	0.63	0.76	0.76	0.76	0.76	0.67	0.67	0.67	0.41	0.43	0.00											
Marechal Foch	0.54	0.54	0.54	0.56	0.56	0.56	0.56	0.56	0.54	0.54	0.54	0.54	0.52	0.52	0.52	0.48	0.56	0.54	0.00										
Zintandel	0.75	0.75	0.75	0.88	0.88	0.78	0.78	0.78	0.80	0.80	0.80	0.80	0.71	0.71	0.71	0.73	0.75	0.75	0.52	0.00									
Niagara	0.69	0.69	0.69	0.82	0.82	0.73	0.73	0.73	0.67	0.67	0.67	0.67	0.73	0.73	0.73	0.75	0.76	0.80	0.58	0.65	0.00								
Carbernet Franc	0.82	0.82	0.82	0.84	0.84	0.86	0.86	0.86	0.80	0.80	0.80	0.80	0.71	0.71	0.71	0.69	0.75	0.75	0.62	0.71	0.73	0.00							
Rubired	0.82	0.82	0.82	0.92	0.92	0.90	0.90	0.90	0.84	0.84	0.84	0.84	0.71	0.71	0.71	0.57	0.63	0.67	0.56	0.67	0.84	0.43	0.00						
Babera	0.53	0.53	0.53	0.75	0.75	0.69	0.69	0.69	0.75	0.75	0.75	0.75	0.57	0.57	0.57	0.71	0.76	0.73	0.50	0.49	0.75	0.80	0.69	0.00					
Macabau Blanc	0.57	0.57	0.57	0.86	0.86	0.76	0.76	0.76	0.78	0.78	0.78	0.78	0.65	0.65	0.65	0.55	0.65	0.61	0.52	0.61	0.82	0.69	0.61	0.47	0.00				
Faustino	0.76	0.76	0.76	0.98	0.98	0.92	0.92	0.92	0.86	0.86	0.86	0.86	0.69	0.69	0.69	0.71	0.76	0.73	0.58	0.80	0.75	0.69	0.69	0.71	0.59	0.00			
Deraware	0.82	0.82	0.82	1.00	1.00	0.82	0.82	0.82	0.96	0.96	0.96	0.96	0.78	0.78	0.78	0.84	0.90	0.75	0.62	0.78	0.80	0.71	0.86	0.76	0.69	0.65	0.00		
Merlot	0.69	0.69	0.69	0.63	0.63	0.69	0.69	0.69	0.75	0.75	0.75	0.75	0.61	0.61	0.61	0.67	0.69	0.73	0.52	0.69	0.78	0.61	0.80	0.67	0.71	0.71	0.69	0.00	
Pinut Noir	0.61	0.61	0.61	0.82	0.82	0.69	0.69	0.69	0.71	0.71	0.71	0.71	0.69	0.69	0.69	0.51	0.61	0.61	0.54	0.80	0.86	0.84	0.80	0.67	0.55	0.86	0.80	0.55	0.00

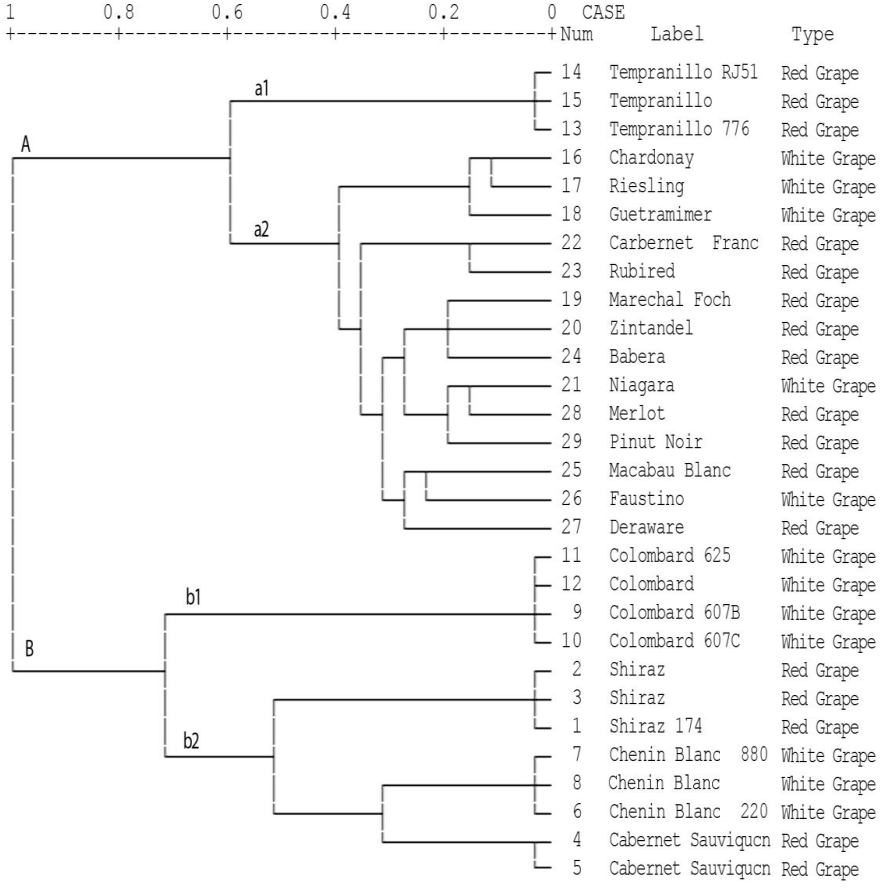

Figure 1. Dendrogram of twenty-nine grapevine varieties obtained using 18 microsatellite primer.

Group a2 consist with 14 samples of red and white grape which are Chardonay, Riesling, Guetramimer, Zintandel, Niagara, Carbernet Franc, Rubired, Babera, Macabau Blanc, Faustino, Delaware, Merlot, Pinut Noir and Marechal Foch, were collected from Suranaree University of Technology, Nakhon Ratchasima province. The genetic relationships of thousand grape varieties in group a2 shown average of 0.4, they were divided into five sub-groups as follows: sub-groups one, consist with 3 samples of a white grape were Chardonay, Riesling and Guetramimer (Figure 1). The genetic distance between Chardonay and Riesling, Chardonay and Guetramimer, and Riesling and Guetramimer showed 0.29, 0.41 and 0.43 (Table 4), respectively. Sub-groups two, consist with 2 samples of a red grape were Carbernet Franc and Rubired. The genetic distance showed 0.43 (Table 4). Sub-groups three, consist with 3 samples of a red grape were Marechal Foch, Zintandel, Babera with the genetic distance among in this subgroup showed between 0.49 - 0.52 (Table 4). For sub-groups four, consist with 3 samples of two red grapes and one white grape were Niagara, Merlot and Pinut Noir,

the genetic distance in this sub-group showed as 0.54 - 0.64 (Table 4). The last sub group of a2, consist with 3 samples; Macabau Blanc, Faustino and Deraware. The genetic distance 0.58 (Table 4) was obtained from Macabau Blanc and Faustino while, the genetic distance 0.68 (Table 4) shown between Macabau Blanc and Deraware. Faustino and Deraware, the genetic distance showed 0.64 (Table 4), showed different SSR profiles and were placed far from the members of this group b2 in the dendrogram (Figure 1).

Group b1 consist has only white grape 4 samples; Colombard was collected from PB Valley Khao Yai Winery, Nakhon Ratchasima province; Colombard 625, Colombard 607B and Colombard 607C were collected from Hua Hin Hills Vineyard, Prachuap Khiri Khan province. The four varieties of grape in this group have no genetic differentiation by using 18 primer pairs of microsatellite marker. The genetic distance showed 0.00 (Table 4), while the genetic relationships of thousand grape varieties in group b1 and b2 average of 0.5 and 0.7 (Figure 1), respectively. It is possible that all of four varieties are the same species, which are genetically distinct from other varieties group. For groups b2 consist with eight samples of both red and white grape; Shiraz, Chenin Blanc and Cabernet Sauvignon were collected from PB Valley Khao Yai Winery, Nakhon Ratchasima province; Shiraz, Shiraz 174, Cabernet Sauvignon, Chenin Blanc 220, Chenin Blanc 880 were collected from Hua Hin Hills Vineyard, Prachuap Khiri Khan province. In this group divided into three sub-groups as follows (Figure 1): subgroups one, consist with three samples of a red grape were Shiraz, Shiraz and Shiraz 174, the three samples have no genetic differentiation by using 18 primer pairs of microsatellite marker. The genetic distance showed 0.00 (Table 4), subgroups two, consist with Chenin Blanc 880, Chenni Blance and Chenin Blanc 220. The three samples are a white grape. The genetic distance among three samples showed 0.00 (Table 4). For subgroups three has two samples of a red grape; Cabernet Sauvignon and Cabernet Sauvignon. The genetic distance showed 0.00 (Table 4), respectively. They were thought to be members of the same group, showed different SSR profiles and were placed far from the members of these groups b1 and b2 in the dendrogram (Table 4 and Figure 1).

This results according to Dzhambazova et al. (5) demonstrated that the genetic structural characterization of wild and varieties Bulgarian grape germplasm was analyzed with microsatellite loci; VVS2, VVMD5, VVMD7, VVMD27, ZAG21, ssrVrZAG62 and ssrVrZAG79. The dendrogram based on the proportion of shared alleles was constructed. The Bulgarian grapevines were clustered into two main groups; group I consist of wild and varieties of grapevine and group II consist of only Danube, wild grapevine.

4. Conclusion

Thirty five microsatellite primer pairs were selected to be used for microsatellite markers to identify 29 grapevine varieties. DNA of grapevines were best amplified with 18 microsatellite primer pairs. The polymorphic band patterns among 29 grapevine varieties were used to cluster analysis using unweighted pair group method with arithmetic mean (UPGMA) based on genetic similarity. The similarity index ranged from 0.9 matrix showed that

the 29 grapevine varieties were clustered into two major groups. This research indicated that the microsatellite markers are useful for identification and analysis of genetic diversity of grapevine and also for grapevine breeding in the future.

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References

(1) Agrawal, GK., Pandey RN and Agrawal VP. Isolation of DNA from Chkerrospondias asillari. BioLect. Biodiv. Lett. 1992; 2: 19-24.

(2) Anonymous. GeneScan Reference Guide Chemistry Reference for The ABI Prism™ 377 Genetic Analyzer PE Applied Biosystems. Division of Perkin-Elmer. International Union of Pure and Applied Chemistry. 1997; pp 1-33.

(3) Bowers J., Dangl G., Vignani R., and Meredith C. DNA isolation and characterization of new polymorphic simple sequence repeat loci in grape (Vitis vinifera L.). Genome. 1996; 39. 628-633.

(4) Bowers JE., Dangl GS., Meredith CP., 1999. Development and characterization of additional microsatellite DNA markers for grape. Am J Enol Viticult 50: 243–246.

(5) Dzhambazova T., Tsvetkov I., Atanassov I., Rusanov K., Martinez Zapater JM., Atanassov A.and Hvarleva T. Genetic diversity in native Bulgarian grapevine germplasm (Vitis vinifera L.) based on nuclear and chloroplast microsatellite polymorphisms. Vitis. 2009; 48 (3), 115–121.

(6) Eiras-Dias JE., Pereira CA and Baptista da Cunha JP. Catalogo das Castas. Regiao do Ribatejo, Oeste e Peninsula de Setubal. Instituto da Vinha e do Vinho, Estacao dVitivinicola Nacional 1988.

(7) Garcia-Beneytez, E., Moreno-Arribas MV., Borrego J., Polo MC and Ibanez J. Application of a DNA Analysis Method for the Cultivar Identification of Grape Musts and Experimental and Commercial Wines of Vitis vinifera L. Using Microsatellite Markers. J. Agric. Food Chem. 2002; 50(21): 6090-6096.

(8) Huang, H., Lu J., Ren Z., Hunter W., Dowd E and Dang P. Mining and validating grape (*Vitis* L.) ESTs to develop EST-SSR markers for genotyping and mapping**.** Mol Breed**.** 2010; 28(2): 241-254.

(9) Nilnond. S. Grape production in Thailand. In M.K. Papademetriou and F.J. Dent, eds.

(10) Grape production in the Asia-Pacific Region. FAO Regional Office for Asia and the Pacific, Bangkok, Thailand. 2001; pp. 70-79.

(10) Sefc K., Regner F., Turetschek E., Glossl J and Steinkellner H. Identification of microsatellite sequences in Vitis riparia and their applicability for genotyping of different Vitis species. Genome . 1999; 42: 367-373.

(11) This P., Jung A., Boccaci P., Borrego J., Botta R., Costantini L.,Crespan M., Dangl GS., Eisenheld C., Ferreira-Monteiro F.,Grando S., Ibanez J., Lacombe T., Laucou V., Magalhaes R., Meredith CP., Milani N., Peterlunger E., Regner F., Zulini L and Maul E. Development of a standard set of microsatellite reference alleles for identification of grape cultivars. Theor. Appl. Genet. 2004; 109: 1448-1458.

(12) Thome, MR and Scott NS. Microsatellite repeats in grapevine revcal DNA polymorphisms wwhen analyzed as sequence-tagged sites (STSs). Theor. Appl. Genet. 1993; 86: 985-990.