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Characterization and comparison of EST-SSR and TRAP markers for genetic analysis of the Japanese persimmon *Diospyros kaki*

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ABSTRACT. We developed and characterized expressed sequence tags (ESTs)-simple sequence repeats (SSRs) and targeted region amplified polymorphism (TRAP) markers to examine genetic relationships in the persimmon genus *Diospyros* gene pool. In total, we characterized 14 EST-SSR primer pairs and 36 TRAP primer combinations, which were amplified across 20 germplasms of 4 species in the genus *Diospyros*. We used various genetic parameters, including effective multiplex ratio (EMR), diversity index (DI), and marker index (MI), to test the utility of these markers. TRAP markers gave higher EMR (24.85) but lower DI (0.33), compared to EST-SSRs (EMR = 3.65, DI = 0.34). TRAP gave a very high MI (8.08), which was about 8 times than the MI of EST-SSR (1.25). These markers were utilized for phylogenetic inference of 20 genotypes of *Diospyros kaki* Thunb. and allied species, with a result that all *kaki* genotypes clustered closely and 3 allied species formed an independent group. These markers could be further exploited for large-scale genetic relationship inference.

Key words: Expressed sequence tags-simple sequence repeats; Targeted region amplified polymorphism; *Diospyros kaki* Thunb.; Genetic relationship

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INTRODUCTION

In recent years, *in silico* approaches have been effectively utilized to develop and characterize the functional genomic markers amplifying genic regions. These markers provide a functional insight into gene-based diversity analysis. Expressed sequence tags (ESTs) have been effectively utilized for the development of these markers and have been subsequently used in the characterization of germplasm (Lima et al., 2010; Kumar Yadav et al., 2011). Simple sequence repeats (SSRs), also called microsatellites, are repetitive stretches of DNA varying from 1-6 bp in length, whereas targeted region amplified polymorphism (TRAP) uses the anchored and arbitrary primers to target the functional gene regions (Hu and Vick, 2003). Essentially, it derives an 18-mer primer from the EST and pairs it with an arbitrary primer that targets the intronic and/or exonic region (AT- or GC-rich core) (Li and Quiros, 2001).

ESTs present a novel way of developing SSRs and TRAP markers in a less time-consuming way and finding more practical applicability in the inference of genetic diversity and linking traits. The distinguishing features of SSR markers and their suitability in the genotyping of populations lie in many factors, namely their high information content, codominant inheritance, high reproducibility, and even distribution along chromosomes (Powell et al., 1996a). EST-derived markers have been well documented and applied in characterization that involves mainly the identification of QTL of important agronomic traits, genetic mapping, genetic diversity, and comparative genomics (Liu et al., 2005; Miklas et al., 2006; Alwala et al., 2006; Hu et al., 2007; Cloutier et al., 2009; Durand et al., 2010). In short, they have been widely used in the study of model fruit plants such as citrus, apple, and cucumber (Chen et al., 2008; Gasic et al., 2009; Hu et al., 2010).

Japanese persimmon (or Oriental persimmon, *Diospyros kaki* Thunb.) is a major commercial and deciduous fruit tree that is believed to have originated in China (Yakushiji and Nakatsuka, 2007). In recent years, several molecular markers were exploited for genetic analysis of *Diospyros* genus. Guo and Luo (2006, 2008, 2011) developed several SSR primers using ISSR-suppression PCR and biotinylated $(GA)_{10}$ and streptavidin-coated magnetic beads in persimmon. Soriano et al. (2006) exploited 37 SSR markers from an enriched genomic library. Du et al. (2009a) compared 4 molecular markers (IRAP, REMAP, SSAP, and AFLP) for genetic analysis in *Diospyros*. However, information is lacking on development of EST-SSR and TRAP in persimmon. In an earlier study, we effectively obtained information on EST-SSR, SNP, and SSR-FDMs using the 9467 *D. kaki* Thunb. ESTs recently reported (Sablok et al. 2011). In the present study, we focused on the identification and characterization of the primer pairs of EST-SSR and TRAP that are able to delineate and infer genotyping in *D. kaki*.

MATERIAL AND METHODS

Plant materials and DNA isolation

A total of 20 genotypes of *Diospyros* spp were used for characterization and included 7 Chinese, 10 Japanese (*D. kaki* Thunb.) and 3 related species (Table 1). All samples were collected from different sites and are currently preserved at the Persimmon Repository, Huazhong Agricultural University, Wuhan, China. Genomic DNA isolation was standardized using fresh leaves and the method proposed by Doyle and Doyle (1990).

Table 1. List of 20 genotypes of *Diospyros* spp used in the present study.

Code	Accessions	Species	Ploidy	Astringent type*	Origin
1	Eshi 1	*D. kaki* Thunb.	2n = 6x = 90	PCNA	China
2	Sifang-tianshi	*D. kaki* Thunb.	2n = 6x = 90	PCNA	China
3	Baogai-tianshi	*D. kaki* Thunb.	2n = 6x = 90	PCNA	China
4	Tongpenshi	*D. kaki* Thunb.	2n = 6x = 90	PCA	China
5	Damopan	*D. kaki* Thunb.	2n = 6x = 90	PCA	China
6	Yunyang-dongshi	*D. kaki* Thunb.	2n = 6x = 90	PCA	China
7	Xiangxi-tianshi	*D. kaki* Thunb.	2n = 6x = 90	PCNA	Japan
8	Huashi 1	*D. kaki* Thunb.	2n = 6x = 90	PVA	Japan
9	Fuyuu	*D. kaki* Thunb.	2n = 6x = 90	PCNA	Japan
10	Maekawa-Jirou	*D. kaki* Thunb.	2n = 6x = 90	PCNA	Japan
11	Hana-gosho	*D. kaki* Thunb.	2n = 6x = 90	PCNA	Japan
12	Oku-gosho	*D. kaki* Thunb.	2n = 6x = 90	PCNA	Japan
13	Youhou	*D. kaki* Thunb.	2n = 6x = 90	PCNA	Japan
14	Nishimura-wase	*D. kaki* Thunb.	2n = 6x = 90	PVNA	Japan
15	Akagaki	*D. kaki* Thunb.	2n = 6x = 90	PVNA	Japan
16	Hiratane-nashi	*D. kaki* Thunb.	2n = 9x = 135	PVA	Japan
17	Chekiang persimmon	*D. glaucifolia* Metc.	2n = 2x = 30	-	China
18	Suruga	*D. kaki* Thunb.	2n = 6x = 90	PCNA	Japan
19	Oily persimmon	*D. oleifera* Cheng	2n = 2x = 30	-	China
20	Date plum	*D. lotus* L.	2n = 2x = 30	-	China

*Persimmon can be classified into four types depending on the nature of the fruit's astringency loss at maturity and the change in flesh color: PCNA = pollination constant non-astringent; PCA = pollination-constant astringent; PVA = pollination-variant astringent; PVNA = pollination-variant non-astringent.

EST-SSR marker development

The EST assembly and EST-SSR primers are described in our previous study (Sablok et al., 2011). PCR amplification were performed using PCRs (20 µL) that contained 20 ng genomic DNA, 100 µM dNTPs, 1X buffer, 1.5 mM $MgCl_2$, 1 U *Taq* DNA polymerase, and 0.8 µM of each primer. The PCR thermal profile involved denaturation at 94°C for 2 min, followed by 35 cycles of 94°C for 45 s, 55°-60°C (primer melting temperature standardization according to GC of primer) for 1 min, 72°C for 75 s, and a final 5-min extension step at 72°C on a Biometra T-Professional PCR (Germany). PCR fragments were separated on 6% denaturing polyacrylamide gels with a constant power at 60 W followed by silver staining for visualization of the amplicons according to the protocol described by Bassam et al. (1991) with slight modification according to Charters et al. (1996).

TRAP marker development

For the development of TRAP markers, 6 fixed primers were derived from persimmon ESTs that were selected for homology to proanthocyanidin metabolism-related genes of other plant species using the TBLASTX program, by comparing the sequences against the non-redundant NCBI nucleotide database with a cut-off E value of 10^{-60} and 80% minimum identity score. Primers were designed using Primer Premier 5.0 (Lalitha, 2000). The 6 arbitrary primers selected for the present study were taken from Li and Quiros (2001). PCR amplification was performed in 15 µL PCR mixtures containing 40 ng genomic DNA, 1X reaction buffer, 1.7 mM $MgCl_2$, 0.3 mM dNTPs, 0.1 µM arbitrary primer, 0.5 µM fixed primer, and 1.5 U *Taq* DNA polymerase. PCR was performed by initially denaturing template DNA at 94°C for 4 min; then, 5 cycles at 94°C for 45 s, 40°C for 45 s, and 72°C for 1 min; followed by 35 cycles at 94°C for 45 s, 52°C (dependent on primer melting temperature) for 45 s, and 72°C for 1 min;

and a final extension step at 72°C for 7 min. PCR fragments were separated on 6% denaturing polyacrylamide gels with a constant power at 60 W by the same method as for EST-SSR.

Data collection and analysis

EST-SSR and TRAP profiles were scored for the presence and absence of the bands as 1 and 0, respectively, in all genotypes. Each polymorphic DNA band at a particular position on the gel was treated as a separate character and scored as allele size. Only robust bands were scored, while ambiguous bands were ignored and excluded from the analysis. To infer the suitability of the proposed marker system diversity index (DI), effective multiplex ratio (EMR; number of polymorphic products from a single amplification reaction), and marker index (MI; the product of EMR and DI) were calculated for each marker according to Powell et al. (1996b) and Geuna et al. (2003).

Genetic similarity values based on the Dice similarity coefficient were calculated, and cluster analysis was performed to construct dendrograms using the unweighted pair-group method with arithmetic averages (UPGMA) and the SAHN clustering modules. Correlation among the different matrices produced by different marker systems was done using the MXCOMP module of the Mantel test. Goodness-of-fit was calculated by the COPH and MXCOMP programs. All analyses were performed with the NTSYSpc 2.10e software (Rohlf, 2000).

RESULTS

EST-SSR and TRAP analysis

Of the 116 designed EST-SSR primers, 71 showed robust PCR amplification and 14 of them revealed polymorphism among 20 persimmon genotypes, indicating a polymorphic rate of 19.72% of the primers for characterization and genotyping of *Diospyros* genus. The sequences and amplification of these polymorphic EST-SSR primers are shown in Table 2, and a sample amplification profile of the EST-SSRs is displayed in Figure 1. The number of alleles detected by the 14 SSR loci ranged from 4 to 14 with an average of 7.86. The percent of polymorphic bands ranged from 83.33 to 100% with an average of 97.42% in 14 pairs of primers.

A total of 36 TRAP primer combinations revealed 2184 PCR amplicons, of which 2072 PCR amplicons were found polymorphic, showing a high percentage of polymorphism (94.87%). A representative gel profile of the TRAP markers is shown in Figure 2. The individual number of bands detected by individual primer combinations varied from 33 (DK6+EM3) to 110 (DK7+EM1) with an average of 60.67. These two primer combinations were also responsible for the minimum (31 in DK6+EM3) and the maximum (108 in DK7+EM1) number of polymorphic bands produced for an average of 57.56 polymorphic bands per primer combination (Tables 3 and 4).

EST-SSR markers versus TRAP markers

A comparative analysis was done within the marker system and the results are summarized in Table 4. We found that TRAP markers showed a higher percentage of amplicon profiling (2184 amplicons) compared to EST-SSR (118 amplicons), but the percentage of polymorphism as

Table 2. Characteristics of the 14 polymorphic expressed sequence tags-simple sequence repeats (ESTs-SSRs) in *Diospyros* L.

Primer code	GenBank accession No.	Repeat motif	Primer sequence (5'-3')	Ta (°C)	Size of expected PCR product (bp)	Total No. of bands	Total No. of polymorphic bands	Percent of polymorphic bands	Resolving power
6615	DC585737	$(CTT)_7$	F: ACACTCCACTCTACCCAAATACC R: GACATCATAAGTCAAAGCACGAA	55	229	9	9	100.00	7.52
4122	DC592784	$(TAGC)_3$	F: ACTCCACCACTTCCTCTTTCAC R: CCCTCTGGCTCTGCTACATTTT	55	205	4	4	100.00	0.95
5553	DC585710	$(GTAGTG)_3$	F: CCAGTTGATGGCAATGGGAGGC R: GGTGCGATGTTGGAGGGAAGAG	58	223	8	8	100.00	3.24
5845	DC585550	$(CCCT)_3$	F: AAACTGCGTCTTCTTCTTGCGATTC R: CGGGTGCTCCATCACTTCTTCA	58	173	7	7	100.00	1.81
8917	DC591591	$(AT)_{10}$	F: ACACGTTCAGTACCATGAGGGA R: AGTACCACAAACCACCAGTGG	55	163	14	14	100.00	7.81
785	DC592314	$(AG)_9$	F: AGTATATCAGCTCACCCAATGC R: TCTTCGAGATCGCGCCTAGAG	50	282	9	9	100.00	3.52
6665	DC592790	$(TA)_9$	F: TGACCAACCCCAAAGTGTGGGAG R: AGGTCCCTCTGGTGAGCACATGC	60	180	8	8	100.00	5.05
1430	DC588341	$(GAG)_5$	F: TCAGTAAAGCTGCGGGCATC R: ACGGTTCTCCTGATCCTCACG	60	270	9	8	88.89	5.91
460	DC589080	$(AT)_{12}$	F: CACAACAGTGAGACAGCCAACTG R: ACAAACCACCAGTGGCCGGAG	55	189	10	10	100.00	3.71
6898	DC584386	$(CT)_9$	F: TGAAGAGATAAGCCTTGCCATGC R: GGGAGGCTATTTGTGGTGACTC	55	130	12	11	91.67	4.29
4379	DC585084	$(GAG)_9$	F: TGACTCTGCTCCACAGGCACTTC R: CTCGTCTGGCAATTCTGCTTCG	58	205	8	8	100.00	4.10
1554	DC586537	$(CAT)_6$	F: CACCGCATCCTCTTCGACATCC R: ACGCATCCGTCAAATCACAACA	55	190	7	7	100.00	4.19
8125	DC592401	$(GGC)_4$	F: TTATCCCATCAAAGCAACCCAC R: CTGCCAACTTCTTCTCCATCTCC	58	185	7	7	100.00	4.29
9004	DC591297	$(GCAGGA)_3$	F: GCCACAAACTTCACAGAGGACC R: AGGCGAGTGCGAGTAAGACGAA	55	243	6	5	83.33	1.52

EST GenBank accession number that contain SSR. F = forward; R = reverse; Ta = annealing temperature.

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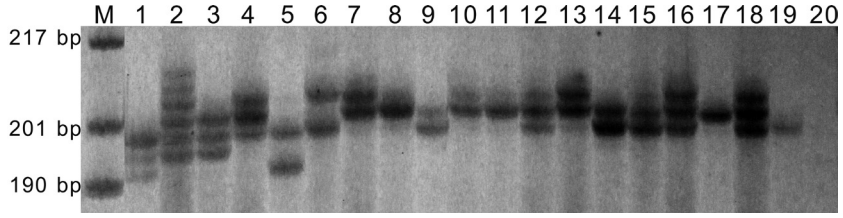

Figure 1. Profile of expressed sequence tag-simple sequence repeat (EST-SSR) amplification using primer 4379. *Lanes 1* to *20* = persimmon genotypes Eshi 1, Sifang-tianshi, Baogai-tianshi, Tongpenshi, Damopan, Yunyang-dongshi, Xiangxi-tianshi, Huashi 1, Fuyuu, Maekawa-Jirou, Hana-gosho, Oku-gosho, Youhou, Nishimura-wase, Akagaki, Hiratane-nashi, Chekiang persimmon, Suruga, Oily persimmon, and Date plum, respectively. *Lane M* = pBR322 DNA/*Msp*I marker.

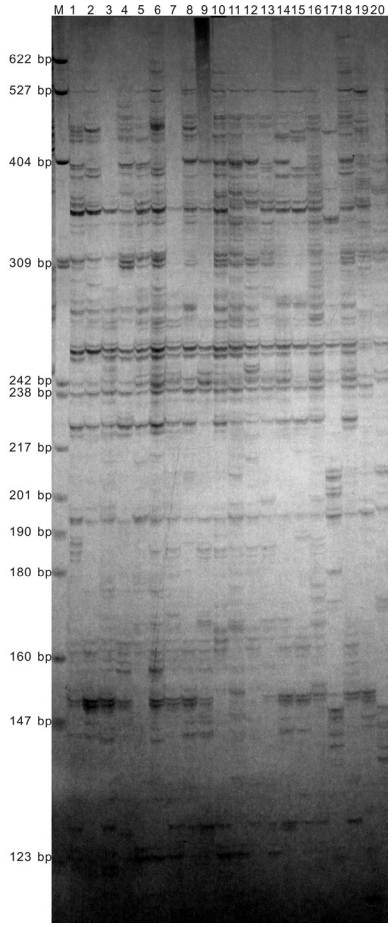

Figure 2. Profile of targeted region amplified polymorphism (TRAP) markers using the DK7+Em1 primer combination. *Lanes 1* to *20* = persimmon genotypes Eshi 1, Sifang-tianshi, Baogai-tianshi, Tongpenshi, Damopan, Yunyang-dongshi, Xiangxi-tianshi, Huashi 1, Fuyuu, Maekawa-Jirou, Hana-gosho, Oku-gosho, Youhou, Nishimura-wase, Akagaki, Hiratane-nashi, Chekiang persimmon, Suruga, Oily persimmon, and Date plum, respectively. *Lane M* = pBR322 DNA/*Msp*I marker.

Table 3. Characteristics of targeted region amplified polymorphism (TRAP) primers in *Diospyros* L.

Fixed primer sequence	Name of target gene	Ta (°C)	GenBank accession No.	Arbitrary primers*	Total No. of bands	Total No. of polymorphic bands	Percent of polymorphic bands	Resolving power
DK5 GCCCACTTCT-ACTGGTTCT	Anthocyanidin reductase	51.2	DC586464	Em1	43	43	100.00	22.29
				Em2	39	37	94.87	16.76
				Em3	39	37	94.87	14.38
				Em4	66	62	93.94	31.05
				Em5	56	50	89.29	25.14
				Em6	46	44	95.65	22.29
DK6 GGGACCCTG-ACAATAAGAA	Anthocyanidin reductase	51.9	DC586464	Em1	58	57	98.28	30.19
				Em2	34	32	94.12	12.95
				Em3	33	31	93.94	14.86
				Em4	70	66	94.29	36.29
				Em5	84	83	98.81	41.52
				Em6	65	65	100.00	32.10
DK7 GAGGACGCA-TTGCTTACA	MYB transcription factor	52	DC587740	Em1	110	108	98.18	50.667
				Em2	54	50	92.59	19.71
				Em3	84	80	95.24	38.29
				Em4	45	39	86.67	15.05
				Em5	50	48	96.00	21.05
				Em6	49	45	91.84	19.43
DK8 CGATGTGGA-AAGAGTTGC	MYB transcription factor	51.1	DC587740	Em1	78	74	94.87	40.19
				Em2	54	49	90.74	20.38
				Em3	64	59	92.19	26.77
				Em4	58	57	98.28	24.29
				Em5	57	51	89.47	22.57
				Em6	42	41	97.62	19.52
DK9 CAGGTTTAC-GATTCAAGGC	Laccase	53.1	DC585535	Em1	79	76	96.20	32.48
				Em2	55	48	87.27	23.14
				Em3	64	59	92.19	28.86
				Em4	77	66	85.71	31.14
				Em5	99	95	95.96	43.71
				Em6	90	84	93.33	36.86
DK10 CTCTTCCTT-TCCATTCCC	Laccase	51.3	DC585535	Em1	70	70	100.00	32.86
				Em2	88	88	100.00	46.10
				Em3	59	59	100.00	30.10
				Em4	53	48	90.57	25.90
				Em5	33	33	100.00	17.33
				Em6	39	38	97.44	19.81

*Arbitrary reverse primer sequences were obtained from Li and Quiros (2001).

Table 4. Levels of polymorphism and comparison of the marker information obtained with expressed sequence tag-simple sequence repeat (EST-SSR) and targeted region amplified polymorphism (TRAP) markers in 20 genotypes.

	Total number of bands	Total No. of polymorphic bands (%)	Average No. of total bands/ assay unit	Average No. of polymorphic bands/ assay unit	DI[a]	EMR[a]	MI[a]
EST-SSR (14 primer pairs)	118	97.46	8.43	8.21	0.34	3.65	1.25
TRAP (36 primer pairs)	2184	94.87	60.67	57.56	0.33	24.85	8.08

[a]Powell et al. (1996b). DI = diversity index; EMR = effective multiplex ratio; MI = marker index.

revealed by TRAP markers (94.87%) was lower as compared to EST-SSR (97.46%), which clearly explained the role of EST-SSR as a better suited marker for the genotyping of the *Diospyros* genus. As well known, the utility of a given marker system is a balance between the level of polymorphism detected (DI) and the extent to which an assay can identify multiple polymorphisms. A convenient estimate of marker utility may therefore be devised from the product of information

content and effective multiplex ratio. MIs for the marker system studied are summarized in Table 4. TRAP showed high MI, as much as 8 times higher than that of EST-SSR in *Diospyros*.

Similarity matrices were constructed based on shared allele analyses, and the average genetic similarity between genotypes was found to be lower in the case of SSR markers (0.626) compared to TRAP (0.662). The correspondence between the similarity matrices produced by the two different marker systems was analyzed using the Mantel test (Table 5). We observed a statistically high correlation ($P < 0.01$), which showed that although both markers are effective in genotyping the species, a combined approach (EST-SSR+TRAP) would be significantly more effective in the genotyping and effective discrimination of the species. Dendrograms of 20 persimmon genotypes were constructed using the UPGMA clustering from the similarity coefficient data of EST-SSR and TRAP and the combined data of both markers. In TRAP, the 20 genotypes were generally divided into three clusters. The three related species formed an independent group that fell well outside of the clusters including all of the genotypes of *kaki* species. There were two groups in the *kaki* species cluster, Chinese native genotypes and Japanese native genotypes. Furthermore, the genotypes of known parental origins were clustered closely. 'Eshi 1' and 'Baogai-tianshi', which are both PCNA (pollination-constant non-astringent) originating from China, were clustered closely. However, 'Sifang-tianshi', another Chinese PCNA, fell out of the group of Chinese native genotypes, which was unexpected. 'Hiratane-nashi' is a rare nonaploid, and it was clustered with other normal Japanese hexaploid cultivars with a low similarity coefficient (Figure 3). However, there were some differences in the SSR tree. 'Hiratane-nashi' was clustered with the Chinese native genotypes, 'Fuyuu' was grouped with 'Sifang-tianshi', and 'Eshi 1' did not cluster with 'Baogai-tianshi' (Figure 4). The tree constructed by combination data of both markers was very similar with the TRAP tree.

Table 5. Mantel test correlation coefficients among similarity matrices obtained using targeted region amplified polymorphism (TRAP) and expressed sequence tag-simple sequence repeat (EST-SSR) markers.

	TRAP	EST-SSR	TRAP+EST-SSR
TRAP	-	-	-
EST-SSR	0.902	-	-
TRAP+EST-SSR	0.999	0.915	-

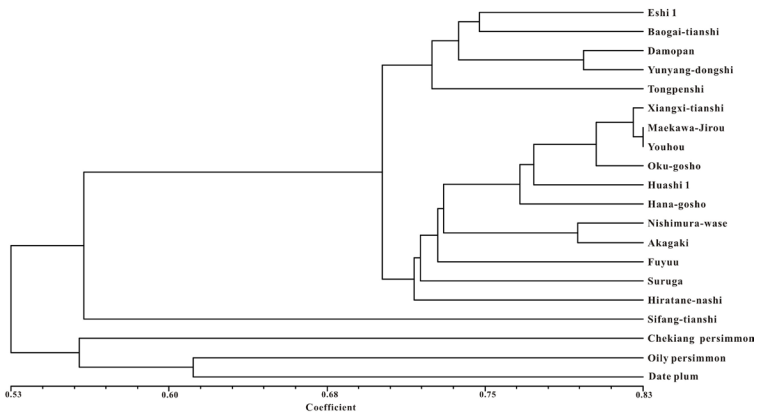

Figure 3. Dendrogram of 20 genotypes from the unweighted pair-group method with arithmetic average cluster analysis based on targeted region amplified polymorphism (TRAP) analysis.

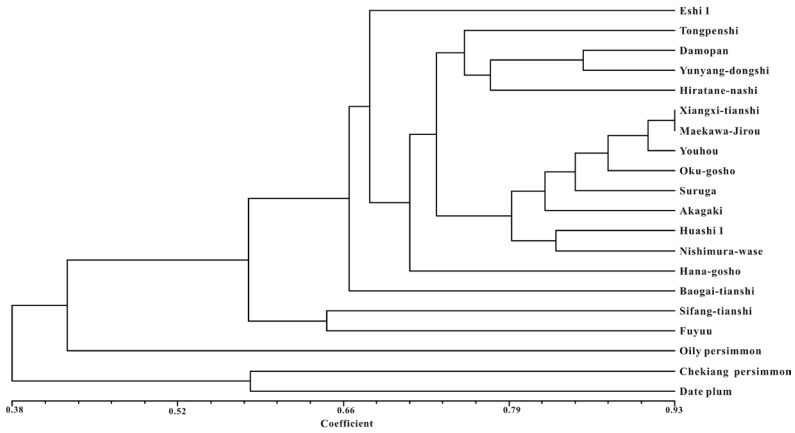

Figure 4. Dendrogram of 20 genotypes from the unweighted pair-group method with arithmetic average cluster analysis based on expressed sequence tag-simple sequence repeat (EST-SSR) analysis.

The cophenetic correlation coefficients between the dendrogram and the original distance matrix for EST-SSR and TRAP were all significant. The values were 0.967 ($P < 0.01$) for EST-SSR and 0.953 ($P < 0.01$) for TRAP.

DISCUSSION

Of the 116 designed EST-SSR primers, 45 (38.79%) did not show clear PCR amplifications in the 20 persimmon genotypes. The reasons for the absence may be due to the presence of a mismatch or the presence of large introns between two primers. Fourteen primer pairs effectively revealed polymorphisms, accounting for 19.72%, higher than those in soybean (12.8%) (Cardle et al., 2000) but lower than those in wheat (25%) and barley (35%) (Thiel et al., 2003). Differences in the polymorphism rates may be partly attributable to the relatedness or the number of genotypes tested. Generally, EST-SSRs show lower levels of polymorphism than genomic SSRs (Eujayl et al., 2002). An earlier report by Guo and Luo (2006) revealed the development of 9 polymorphic (75%) genomic SSR and showed that the number of alleles ranged from 5 to 20 alleles per locus with an average of 13.8. It should be mentioned here that they studied 30 persimmon cultivars. Also, Soriano et al. (2006) reported that 22 polymorphic (59.46%) genomic SSR markers represented from 2 to 8 alleles per locus with an average of 5.14 when estimated in 12 persimmon cultivars. In comparison to the above reports based on analysis of the 20 persimmon genotypes by the 14 polymorphic EST-SSRs (19.72%), the number of alleles detected ranged from 4 to 14 (with an average of 7.86). The difference in the polymorphic level among these studies may result from the nature of the SSRs (genomic versus EST-derived), the relatedness of the genotypes, or simply the number of genotypes tested.

In comparison to the EST-SSR markers, the TRAP technique is a relatively high-throughput PCR-based marker system and very efficient compared to other marker systems, which use arbitrary primers for amplification of the non-coding region of the genome. This marker has been extensively used in spinach to assess the genetic diversity and has shown a significant amount of polymorphism (19.5%) across 38 germplasms and 10 commercial hy-

brids (Hu et al., 2007). Furthermore, the TRAP technique generates a greater number of amplicons, comparable to the AFLP technique. Using 142 *Buxus* genotypes, Van Laere et al. (2011) generated 750 polymorphic AFLP fragments from 3 primer combinations. In comparison, we used varietal and wild-type persimmon to reveal a total of 2072 polymorphic bands from 36 primer pairs with an average of 57.56 polymorphic markers per reaction.

In our study, we effectively demonstrated the development and characterization of EST-SSR primer pairs and TRAP primer combinations for the genus *Diospyros*. We obtained more amplicons in the case of TRAP compared to EST-SSR. Since both markers represent the functional coding region of the genome, this report presents the development of functional candidate markers in genus *Diospyros*. We used MI and its components, DI and EMR, to examine the overall efficiency of the two marker systems as applied to persimmon (Du et al., 2009b). It was useful to analyze each of these components and their ramifications individually. Comparison of the diversity index calculated from the experimental data exhibited that EST-SSR was more polymorphic, which was in agreement with previous research in other plants (Milbourne et al., 1997; McGregor et al., 2000; Belaj et al., 2003; Panwar et al., 2010). However, TRAPs revealed high values of loci per assay unit, average number of polymorphic bands per assay unit, and high EMR, indicating that a large amount of genetic information could be detected using this marker, which was in keeping with the study by Alwala et al. (2006) and Hu et al. (2007). In TRAP, the value of MI produced by DI and EMR was about 7 times higher than that in EST-SSR, indicating that TRAP is a marker system with a good comprehensive performance.

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