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RESEARCH ARTICLE

Differences of EST-SSR and genomic-SSR markers in assessing genetic diversity in poplar

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Abstract We analyzed the genetic differences of 16 poplar clones between genomic-SSR and EST-SSR markers. The statistical results show that the average number of alleles detected by genomic-SSR was 4.1, Shannon's index 1.0646, observed heterozygosity 0.4427 and expected heterozygosity 0.5523, while for the EST-SSR, the average number of alleles was 2.8, Shannon's index 0.6985, observed heterozygosity 0.2330 and expected heterozygosity 0.4684. Cluster analysis indicated that the EST-SSR capacity of genotypic identification was more precise than that of genomic-SSR. These results reveal that EST-SSR and genomic-SSR have statistically significant genetic differences in polymorphism detection and genotypic identification. These differences could provide a theoretical basis for the rational use of SSR markers in species diversity and other related research.

Key words poplar, genomic-SSR, EST-SSR, genetic differences

1 Introduction

Genetic diversity plays an important role in survival and adaptability of species. Methods of evaluation of genetic diversity include phenotypic, biochemical and molecular markers (Sun et al., 1996). Among these markers, molecular markers are not affected by environmental conditions, are found in the entire genome and are, therefore, widely used in studies of genetic diversity. Microsatellites, also referred to as simple sequence repeats (SSRs), are tandem repeated sequences and comprise mono-, di-, tri-, tetra-, penta- or hexa-nucleotide units. SSRs possess a large number of advantages, such as high information content, codominance, locus specificity and easy detection as PCR-based molecular markers. These have become important tools to study genetic diversity, construct genetic maps and analyze evolutionary processes of species (Tuskan et al., 2004; Varshney et al., 2005; Yin et al., 2008). Traditional methods of developing SSR markers are usually time-consuming and labor-intensive. With the development of functional genomics, expressed sequence tag (EST)-SSR has become the latest aspect of SSR development. Sequence information for EST-SSR is readily available and hence, the need of genomic library screening can be avoided. Moreover, EST-SSR originates from transcribed regions of genomes, which may reflect information of specific genes. Being highly conservative, they are transferable between species. Genomic-SSR and EST-SSR are different in assessing genetic diversity of species, because they come from different regions of genomes. This difference was confirmed in wheat (*Triticum aestivum*) and other plants (Yang et al., 2005; Chang et al., 2009; Wen et al., 2010). In addition, these studies showed that genetic differences between genomic-SSR and EST-SSR are very useful in species diversity research.

Poplars (*Populus*) are well known as model trees in forestry research. Assessing genetic diversity of poplars is very important for genetic improvement and selective breeding (Su et al., 2004). Hence, for our study, we selected 16 poplar clones for analyzing the genetic differences of EST-SSR and genomic-SSR in order to lay a basis for related research when different SSR markers are used.

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2 Materials and methods

2.1 Plant material

In order to analyze the difference between EST-SSR and genomic-SSR for assessing genetic diversity in poplar, 16 clones were chosen. The material came from three subgenera of poplar: *Leuce*, *Tacamahaca* and *Aigeiros*. The details are shown in Table 1. Total genomic DNA was extracted from young leaves by a modified CTAB method as described by Dellaporta et al. (1983). The genomic DNA was diluted to a concentration of 25 ng·μL^{-1} for PCR amplification.

2.2 Primer design

A total of 5359 EST sequences were downloaded from NCBI. These sequences were analyzed by an EST-trimmer (http://pgrc.ipk-gatersleben.de) in order to discard the sequences that contain the vectors, poly (A), poly (T) and those EST sequences shorter than 100 bp. The software, SSRIT (http://www.gramene.org/db/searches/ssrtool), was used to scan the SSR structure in these EST sequences. EST-SSR primers were designed by Primer3 software online. Primer parameters included length 18–23 bp, renaturation temperature 53–56°C and G + C content 47.7%–56.2%. In the end, ten EST-SSR primers were selected. Simultaneously, ten genomic-SSR primers were randomly selected from the PMGC database (http://www.ornl.gov/sci/ipgc/ssr_resource.htm).

2.3 PCR amplification and electrophoresis

PCR reactions were performed using the gene-specific primers with *Taq* polymerase in the final volume of 20 μL, containing 13.25 μL of ddH_2O, a 2.0 μL of 10 × buffer, 2.0 μL of 2.0 mmol·L^{-1} dNTP, 0.8 μL of each primer (10 μmol·L^{-1}), 1 μL of template and 0.7 U *Taq* DNA polymerase. Amplifications were carried out at the following cycling parameters: preliminary denaturation (5 min, 94°C), then 30 cycles of denaturation (20 s, 94°C), annealing (30 s, 58°C) and extension (40 s, 72°C), followed by a final extension (10 min, 72°C) and stored at 4°C until used. PCR products were separated on a 6% denaturing polyacrylamide gel.

2.4 Statistical calculations

Polymorphic alleles obtained with each primer pair were scored for their presence (1) or absence (0). The raw data matrix obtained was used to calculate genetic similarity coefficients between clones as follows:

$GS = 2N_{ij}/(N_i + N_j)$

where GS is the genetic similarity coefficient, N_{ij} the number of alleles in common between clones *i* and *j* and N_i and N_j are the total number of alleles observed for clones *i* and *j*, respectively. A dendrogram was constructed based on these genetic similarity coefficients using the UPGMA (unweighted pair group method with arithmetic averages) method with the NTSYS-pc program. Genetic diversity parameters, i.e., observed heterozygosity (H_o), expected heterozygosity (H_e) and Shannon's diversity index (*I*), were calculated using POPGENE version 1.32 (Nei and Li, 1979; Botstein et al., 1980).

3 Results

3.1 Genomic-SSR and EST-SSR polymorphism analysis

Ten genomic-SSR primers and ten EST-SSR primers were used to estimate the genetic diversity of the 16 poplar clones. A total of 69 alleles were identified and all of them were polymorphic (Fig. 1). The size of the amplification products for the genomic-SSR and EST-SSR ranged from 170 to 443 bp. Ten genomic-SSR

Table 1 Poplar clones for SSR analysis

No.	Clone	Species	Source	No.	Clone	Species	Source
1	9	(*P. tomentosa* × *P. bolleana*) × *P. tomentosa*	Beijing	9	I69	*P. deltoides*	Shandong
2	10	(*P. tomentosa* × *P. bolleana*) × *P. tomentosa*	Beijing	10	Pu-1	*P. ussuriensis*	Jilin
3	13	(*P. tomentosa* × *P. bolleana*) × *P. tomentosa*	Beijing	11	135	*P. deltoids* × *P. ussuriensis*	Beijing
4	46	(*P. tomentosa* × *P. bolleana*) × *P. tomentosa*	Beijing	12	136	*P. deltoids* × *P. ussuriensis*	Beijing
5	51	(*P. tomentosa* × *P. bolleana*) × *P. tomentosa*	Beijing	13	139	*P. deltoids* × *P. ussuriensis*	Beijing
6	LM50	*P. tomentosa* 'LM50'	Shandong	14	144	*P. deltoids* × *P. ussuriensis*	Beijing
7	MX	*P. tomentosa* × *P. bolleana*	Beijing	15	147	*P. deltoids* × *P. ussuriensis*	Beijing
8	XY	*P. alba* × *P. glandulosa*	Shandong	16	SN21	*P. szechuanica*	Tibet

primers generated 41 alleles. The number of alleles for each genomic-SSR primer ranged from 2 to 5, with a mean of 4.1. In contrast, the number of alleles for each EST-SSR primer ranged from 2 to 4, with a mean of 2.8. Therefore, more alleles were obtained with the genomic-SSR than with EST-SSR. Genotyping data obtained for polymorphic alleles were used to calculate Shannon's diversity index (Table 2). The Shannon's index based on genomic-SSR data ranged from 0.5454 to 1.6000, with a mean of 1.0646, while those

Table 2 Information of primers and statistical results

Primer	Primer sequence (5′→3′)	Repeat type	Length (bp)	Band number	Linkage group	I	H_o	H_e
ESTCU310415	F: CCCGAGTCAATCTGAGTTAGTA R: CTTGTTTATTGGAGATGGAGC	$(AAAT)_4$	122	3	IV	0.8813	0	0.6133
ESTCU310401	F: GTGCAGGCAGATATTTATGGA R: GGAAGCAGCAGTTGAAGAAG	$(TGCTTC)_3$	127	4	X	1.3800	0.8125	0.7257
ESTCU310140	F: AACCGTATGAAACTTTAGGCA R: AAACCCACCCACTGTTATTG	$(TTTATA)_3$	334	4	IV	0.8571	0	0.6036
ESTCU316835	F: GCAGAGGAAGCAGCAAGAG R: CCAAGTCACGGGACAGTAAAG	$(CTT)_5$	449	2	Scaffold -28	0.4404	0.5000	0.4444
ESTCU306954	F: CCCCGAATATCTCGTCTT R: GGTTTGGTTGGGTTGTCT	$(CGG)_5$	522	2	I	0.2900	0	0.1244
ESTCU307135	F: AAGGTGAGGAGCAGCAGAG R: AAATCAAACCCTAAAGCACAG	$(ATC)_5$	464	3	XII	0.8982	0	0.5562
ESTCU309310	F: TTATCCACCCTCCCTGTCTC R: AAAGGAAAGGTCCATCGTAAT	$(TTTG)_4$	225	3	IX	1.0972	0.4285	0.6888
ESTCU307757	F: AACAATCTCGCAGCAGGAA R: GAAATGTCAGCGTTGGGTC	$(CGG)_5$	358	2	I	0.1168	0	0.2343
ESTCU307720	F: CGAGGGAATGGATGAGATG R: CTTAAATAGAGCCAGGGAAATAC	$(GAGGAA)_3$	390	3	Scaffold -140	0.6018	0.2142	0.3190
ESTCU308303	F: AAGAAAGTGTAGGAGCTGGACC R: GGCAGATCATCACAACGAAAT	$(AAAT)_4$	328	2	II	0.4221	0.3750	0.3750
GenomicPMGC-93	F: ATCATGCGTTCGGCTACAGC R: CTCAAACTCCAACTGTTATAAC	$(CTT)_7$	350	3	I	0.9742	0.0625	0.5712
GenomicPMGC-2826	F: GCTTCTTTAGCGACATGCATC R: GTCAGAACTGTGACAGTAACC	$(GA)_9$	237	4	IV	0.5454	0.2000	0.3578
GenomicORPM-21	F: GGCTGCAGCACCAGAATAAT R: TGCATCCAAAATTTTCCTCTTT	$(AG)_{12}$	206	2	IX	0.5982	0.4000	0.3200
GenomicORPM-344	F: GGAGATTGTCGGAGAATGGA R: TGGACGTTACGATAGGAGTGG	$(TC)_8$	229	5	X	1.6000	0.8660	0.7181
GenomicGCPM-1013	F: TGCTCCACTCAATGTCAATA R: GACGGTGATAAGAGGAACTG	$(TA)_{11}$	207	4		0.9919	0.1818	0.3808
GenomicGCPM-1017	F: GTTTAATTCCCACGTCGTTA R:CGAATGAAGAAAAACCATTC	$(GT)_{11}$	183	5		1.1330	0.6250	0.6960
GenomicGCPM-1019	F: CAGGTCCGTAGCACTATTTC R: GCTCAAATGGACATCAAAGT	$(TAA)_6$	208	5		1.2267	0.5714	0.6787
GenomicGCPM-1353	F: GAAAACTGATTCCTGATTCG R: CAAGAATCAATGCATGTCTG	$(AT)_9$	150	4		0.8016	0.3750	0.4062
GenomicGCPM-136-1	F: TATTGGCAGCAAGAAAGAAT R: TAACTTTTGACATTCCCACC	$(AGG)_6$	154	5		1.3936	0.5625	0.7172
GenomicWPMS-16	F: CTCGTACTATTTCCGATGATGACC R: AGATTATTAGGTGGGCCAAGGACT	$(GTC)_8$	145	4	VII	1.3815	0.5833	0.6779

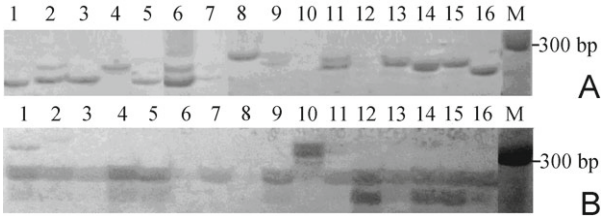

Fig. 1 Amplified results of 16 poplar clones using genomic-SSR (A) and EST-SSR (B) primers. Lane M represents DNA markers and lanes 1–16 represent amplified results of 16 clones.

based on EST-SSR ranged from 0.1168 to 1.3800, with a mean of 0.6985. Furthermore, we carried out an analysis of variance to characterize the difference in the Shannon's index based on EST-SSR and genomic-SSR. The results showed that in assessing genetic diversity of poplar, the difference between genomic-SSR and EST-SSR was statistically significant ($p < 0.01$).

3.2 Correlation analysis of genomic-SSR and EST-SSR similarity coefficients

The similarity coefficients, counted from genomic-SSR data, ranged from 0.3902 to 0.9268, with an average of 0.6623 (Table 3). Among the 16 poplar clones, No. 3 had the smallest similarity coefficients with Nos. 9 and 13, while No. 14 had the largest coefficients with Nos. 11 and 13. For EST-SSR, the similarity coefficients ranged from 0.4118 to 0.9706 with a mean of 0.6668. Among all clones, No. 10 had the smallest similarity coefficients with Nos. 2, 5, 6 and 7, while No. 4 had the largest coefficients with Nos. 3, 5 and 6 and as well No. 11 with Nos. 9, 13 and 15 (Table 4). The calculated similarity coefficients from the 69 SSR data ranged from 0.4400 to 0.9333, with an average of 0.6647 (Tables 3–4). No. 11 had the smallest similarity coefficient with No. 6 and the largest coefficients with Nos. 13 and 14. Clearly, the similarity coefficients between clones, calculated from the data of the two markers, showed significant differences, suggesting that genomic-SSR and EST-SSR vary in their identification of poplar sections.

In the backcross population of (*P. tomentosa* × *P. bolleana*) × *P. tomentosa*, the similarity coefficients between No. 2 and No. 4 were the smallest when calculated from both markers. In the cross-population *P. deltoides* × *P. ussuriensis*, the similarity coefficients between No. 12 and No. 14 were also the smallest. These results suggest that both genomic-SSR and EST-SSR can accurately reflect genetic relationships of different genotypes.

Correlation analysis of similarity coefficients for genomic-SSR, EST-SSR and genomic-SSR + EST-SSR revealed that these similarity coefficients are significantly and positively correlated. The correlation coefficient of EST-SSR with genomic-SSR + EST-SSR was the largest (Table 5), indicating that EST-SSR can more accurately reflect the genetic relationship between genotypes.

Table 3 Genetic similarity coefficients calculated by genomic-SSR and genomic-SSR + EST-SSR in 16 poplar clones

No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	1	0.8933	0.8533	0.8533	0.8667	0.8400	0.8000	0.8133	0.5600	0.5867	0.5467	0.6133	0.5600	0.5867	0.6133	0.6000
2	0.8780	1	0.8800	0.8267	0.8400	0.8667	0.8000	0.7600	0.4800	0.5333	0.4667	0.5600	0.4533	0.4800	0.5600	0.5200
3	0.8049	0.8293	1	0.8933	0.8533	0.9067	0.8133	0.8000	0.4667	0.5200	0.4800	0.5467	0.4667	0.4933	0.5467	0.5333
4	0.8293	0.7561	0.8293	1	0.9067	0.9333	0.8133	0.7733	0.4933	0.5200	0.4533	0.5467	0.4667	0.4933	0.5200	0.5333
5	0.8780	0.8049	0.7805	0.8537	1	0.8667	0.8267	0.7867	0.5067	0.5867	0.5200	0.6400	0.5067	0.5600	0.5867	0.6267
6	0.8293	0.8049	0.8780	0.9024	0.8049	1	0.7467	0.7600	0.4533	0.4800	0.4400	0.5067	0.4533	0.4800	0.5067	0.4933
7	0.7561	0.7317	0.7561	0.7805	0.7805	0.6829	1	0.6667	0.5200	0.5733	0.5067	0.6000	0.5200	0.5733	0.5733	0.5867
8	0.8537	0.7805	0.8049	0.7805	0.8293	0.7805	0.6585	1	0.5333	0.5867	0.5467	0.6133	0.5333	0.5600	0.6133	0.6000
9	0.5366	0.4634	0.3902	0.4634	0.5122	0.4146	0.5366	0.5366	1	0.6800	0.8533	0.7067	0.8933	0.8667	0.8400	0.6933
10	0.6585	0.6341	0.5610	0.5854	0.7317	0.5366	0.7073	0.6585	0.6341	1	0.7733	0.8667	0.7333	0.7333	0.8133	0.6667
11	0.5366	0.4634	0.4390	0.4146	0.5610	0.4146	0.5366	0.5854	0.7561	0.7805	1	0.7733	0.9333	0.9333	0.9067	0.7333
12	0.6341	0.6098	0.5366	0.5610	0.7073	0.5122	0.6341	0.6829	0.6585	0.8780	0.7561	1	0.7333	0.7333	0.8133	0.7733
13	0.5366	0.4146	0.3902	0.4146	0.5122	0.4146	0.5366	0.5366	0.8537	0.6829	0.9024	0.6829	1	0.9200	0.8667	0.6933
14	0.6098	0.4878	0.4634	0.4878	0.5854	0.4878	0.6098	0.6098	0.8293	0.7561	0.9268	0.6585	0.9268	1	0.8933	0.7467
15	0.6341	0.6098	0.5366	0.5122	0.6585	0.5122	0.6341	0.6829	0.7561	0.8780	0.8537	0.8049	0.8049	0.8780	1	0.7733
16	0.6098	0.5366	0.5122	0.5366	0.6829	0.4878	0.5610	0.7073	0.6829	0.7073	0.7317	0.7805	0.6829	0.7561	0.7805	1

Note: genetic similarity coefficients of genomic-SSR are below the main diagonal and those of genomic-SSR + EST-SSR above the main diagonal.

Table 4 Genetic similarity coefficients calculated by EST-SSR and genomic-SSR + EST-SSR in 16 poplar clones

No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	1	0.8933	0.8533	0.8533	0.8667	0.8400	0.8000	0.8133	0.5600	0.5867	0.5467	0.6133	0.5600	0.5867	0.6133	0.6000
2	0.9118	1	0.8800	0.8267	0.8400	0.8667	0.8000	0.7600	0.4800	0.5333	0.4667	0.5600	0.4533	0.4800	0.5600	0.5200
3	0.9118	0.9412	1	0.8933	0.8533	0.9067	0.8133	0.8000	0.4667	0.5200	0.4800	0.5467	0.4667	0.4933	0.5467	0.5333
4	0.8824	0.8529	0.9706	1	0.9067	0.9333	0.8133	0.7733	0.4933	0.5200	0.4533	0.5467	0.4667	0.4933	0.5200	0.5333
5	0.9118	0.8824	0.9412	0.9706	1	0.8667	0.8267	0.7867	0.5067	0.5867	0.5200	0.6400	0.5067	0.5600	0.5867	0.6267
6	0.8529	0.9412	0.9412	0.9706	0.9412	1	0.7467	0.7600	0.4533	0.4800	0.4400	0.5067	0.4533	0.4800	0.5067	0.4933
7	0.8529	0.8824	0.8824	0.8529	0.8824	0.8235	1	0.6667	0.5200	0.5733	0.5067	0.6000	0.5200	0.5733	0.5733	0.5867
8	0.7647	0.7353	0.7941	0.7647	0.7353	0.7353	0.6765	1	0.5333	0.5867	0.5467	0.6133	0.5333	0.5600	0.6133	0.6000
9	0.5882	0.5000	0.5588	0.5294	0.5000	0.5000	0.5000	0.5294	1	0.6800	0.8533	0.7067	0.8933	0.8667	0.8400	0.6933
10	0.5000	0.4118	0.4706	0.4412	0.4118	0.4118	0.4118	0.5000	0.7353	1	0.7733	0.8667	0.7333	0.7333	0.8133	0.6667
11	0.5588	0.4706	0.5294	0.5000	0.4706	0.4706	0.4706	0.5000	0.9706	0.7647	1	0.7733	0.9333	0.9333	0.9067	0.7333
12	0.5882	0.5000	0.5588	0.5294	0.5588	0.5000	0.5588	0.5294	0.7647	0.8529	0.7941	1	0.7333	0.7333	0.8133	0.7733
13	0.5882	0.5000	0.5588	0.5294	0.5000	0.5000	0.5000	0.5294	0.9412	0.7941	0.9706	0.8235	1	0.9200	0.8667	0.6933
14	0.5588	0.4706	0.5294	0.5000	0.5294	0.4706	0.5294	0.5000	0.9118	0.7059	0.9412	0.7041	0.9118	1	0.8933	0.7467
15	0.5882	0.5000	0.5588	0.5294	0.5000	0.5000	0.5000	0.5294	0.9412	0.7353	0.9706	0.8235	0.9412	0.9118	1	0.7733
16	0.5882	0.5000	0.5588	0.5294	0.5588	0.5000	0.6176	0.4706	0.7059	0.6176	0.7353	0.7647	0.7059	0.7353	0.7647	1

Note: genetic similarity coefficients of EST-SSR are below the main diagonal and those of genomic-SSR + EST-SSR above the main diagonal.

Table 5 Correlation between genetic similarity coefficients based on genomic-SSR and EST-SSR

Marker	Genomic-SSR	EST-SSR
Genomic-SSR	1	
EST-SSR	0.96**	
Genomic-SSR + EST-SSR	0.98**	0.99**

Note: **means significant correlation ($p < 0.01$).

3.3 Cluster analysis

On the 0.55 level of genomic-SSR similarity coefficients, the 16 clones were clustered into two groups: group I consisted of LM50 (*P. tomentosa*), MX (*P. tomentosa* × *P. bolleana*), XY (*P. alba* × *P. glandulosa*) and five progenies of MX backcrossed with LM50; group II consisted of I69 (*P. deltoides*), Pu-1 (*P. ussuriensis*), SN21 (*P. szechuanica*), five progenies of I69 crossed with Pu-1 (Fig. 2). On the 0.51 level of EST-SSR similarity coefficients, all clones were clustered into the same two groups (Fig. 3). The results suggest that both genomic-SSR and EST-SSR can distinguish between different poplar genotypes. Moreover, as seen in Figs. 2 and 3, EST-SSR could more accurately differentiate *P. alba* × *P. glandulosa* from other white poplars than genomic-SSR. The clustering result of genomic-SSR + EST-SSR was more similar to that of EST-SSR than to genomic-SSR (Fig. 4). Hence, it can be concluded that EST-SSR can more accurately present genetic relationships between genotypes.

4 Discussion

4.1 Genetic differences between genomic-SSR and EST-SSR

Analyses of the difference of genomic-SSR and EST-SSR in assessing the genetic diversity of the 16 poplar clones revealed that Shannon' index, H_o, H_e, genetic similarity coefficients and polymorphisms of alleles of genomic-SSR were significantly larger than those of EST-SSR, showing that EST-SSR is more conservative than genomic-SSR in poplars. This conservation might result from the hitchhiking effect during the evolution of species (Maynard Smith and Haigh, 1974). These results are consistent with related research in wheat, providing evidence that genetic differences between genomic-SSR and EST-SSR are significant. Thus, an objective evaluation method is a prerequisite to evaluate genetic diversity of species (Jia et al., 2001) and utilizing SSR markers developed from various sources can be more accurate and objective when assessing genetic diversity.

4.2 Characteristics and application of EST-SSR

The high conservation of EST-SSR shows that a considerable degree of transferability exists between related species (Ellis and Burke, 2007; Moccia et al., 2009). Yang et al. (2008) reported that the transferability of poplar EST-SSR in *Salix* was 54.2%, while

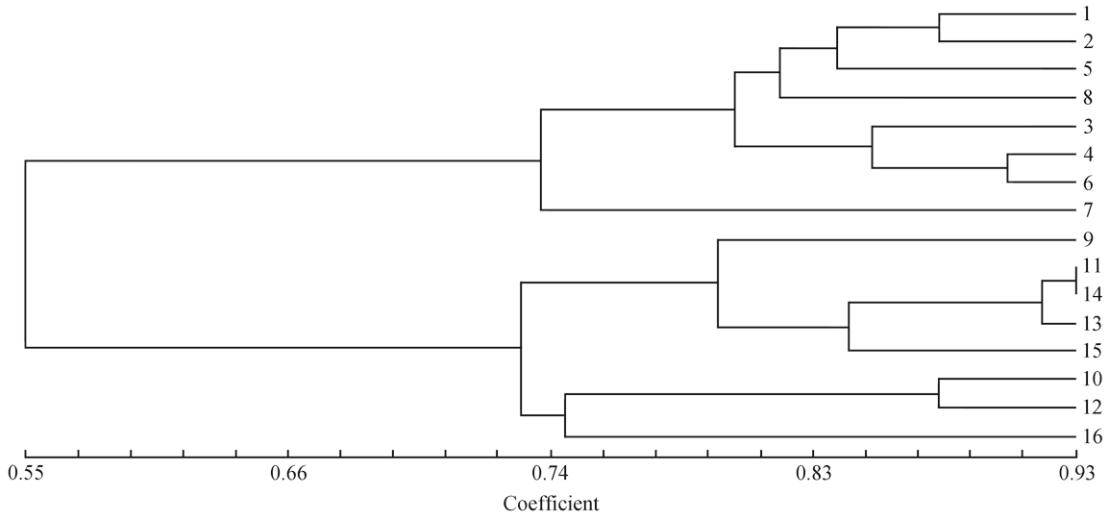

Fig. 2 Dendrogram of genetic similarity coefficients from genomic-SSR of 16 poplar clones

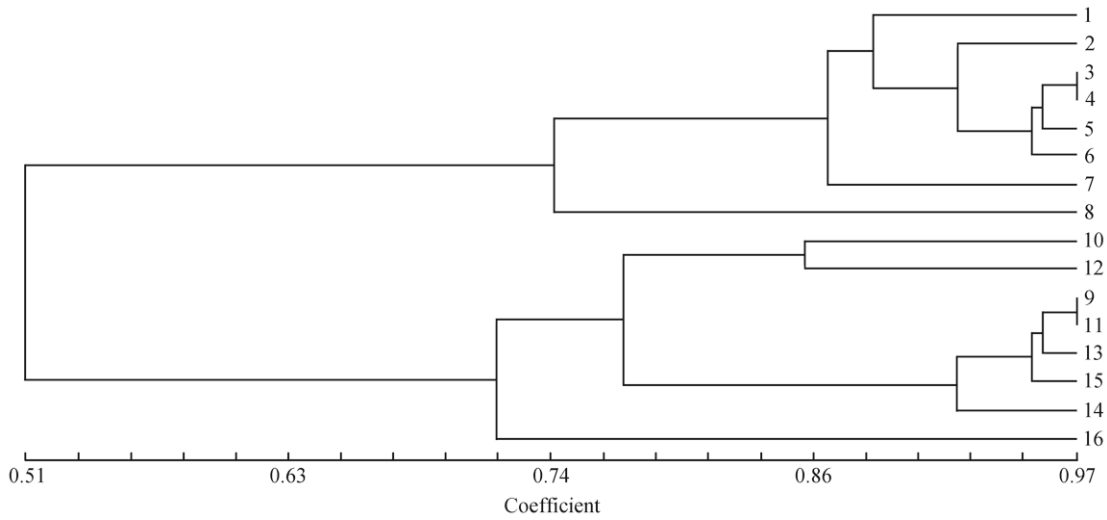

Fig. 3 Dendrogram of genetic similarity coefficients from EST-SSR of 16 poplar clones

only 10.4% for genomic-SSR. Similar results were obtained in *Zoysia japonica* (Zhao et al., 2008) and *Epimedium sagittatum* (Zeng et al., 2010). Our study, therefore, provided another practical demonstration of the advantages of EST-SSR in constructing genetic linkage maps that can be used in comparing related species. Furthermore, comparing genetic linkage maps among related species can provide a new approach in elaboration of phylogenesis in different genera.

The cluster analysis revealed that the ability of EST-SSR to discriminate was more accurate than that of genomic-SSR between species and suitable for the study of genetic differences between species. This is consistent with the finding in durum wheat (*Triticum durum*) (Eujayl et al., 2001). EST-SSR can directly reflect the variation in gene transcriptional regions, closely associated with phenotypic, physiological and biochemical indices as well as with metabolic features. For example, the $(CT)_n$ repeat number of the 5′ untranslated regions of the *Waxy* gene is considered closely related to the starch content in rice (Ayres et al., 1997). Hence, developing research of EST-SSR associated with these characteristics is not only beneficial in the identification of diversity of gene functions, but also lays the foundation for the application of genetic linkage maps in high resolution mapping (Hanai et al., 2010).

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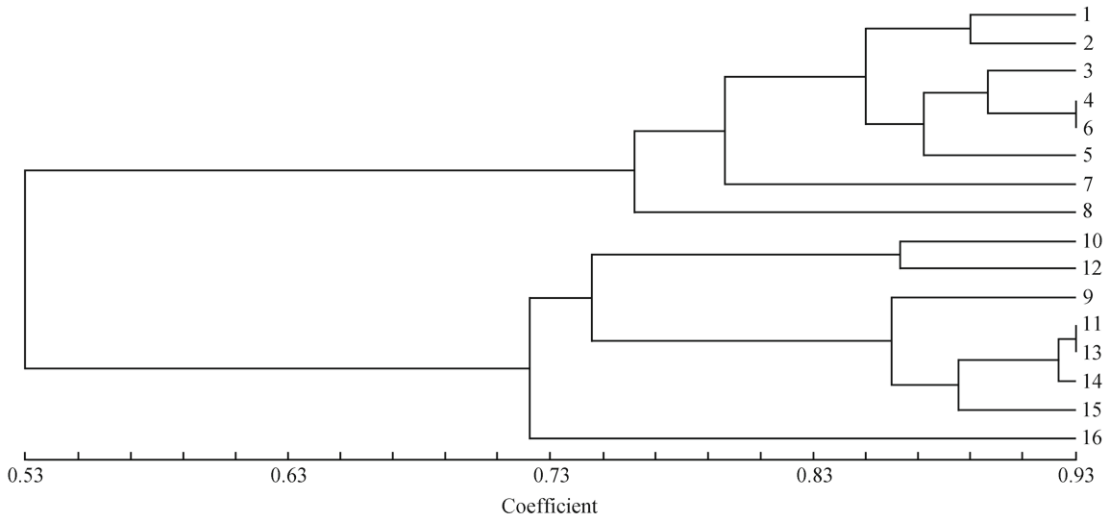

Fig. 4 Dendrogram of GS from genomic-SSR + EST-SSR of 16 poplar clones

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