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Mapped SSR markers unevenly distributed on the cotton chromosomes

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Abstract In the past 15 years, more than 9000 SSR markers have been developed, which are a valuable resource for cotton genetics and breeding. Many of them have been mapped in cotton, and several high-density genetic maps have been constructed. In this paper, we jointly analyzed the mapped SSRs to characterize their motifs on cotton chromosomes. The results showed that SSR loci were distributed unevenly on cotton chromosomes with more on C11 and C19 and less on C02 and C04. Different sources of SSRs contributed different predominant SSR types, which were also distributed unequally on cotton chromosomes. Compared to SSRs derived from enriched libraries and BAC end sequences, EST-SSRs could generate novel SSR motifs especially for AT/TA of di-, tetra-, penta- and hexanucleotide. SSR motifs showed a biased distribution on cotton chromosomes with more on C05, C11, and C19, and less on C02 and C04. The uneven distribution of SSRs on cotton chromosomes would help us to enrich the cotton genetic map by motif-specific SSRs. This analysis gave us new knowledge on the architecture of the cotton genome.

Keywords cotton, SSR, motif, genetic linkage map

1 Introduction

The genus *Gossypium* is comprised of 45 diploid and 5 allotetraploid species (Fryxell, 1992). There are four cultivated species: two are tetraploid, *G. hirsutum* L. [$2n$

$= 4x = 52$, (AD)₁] and *G. barbadense* L. [$2n = 4x = 52$, (AD)₂], and two diploid, *G. herbaceum* L. ($2n = 2x = 26$, A₁) and *G. arboretum* L. ($2n = 2x = 26$, A₂). *G. hirsutum* L. accounts for about 90% of fiber production; while *G. barbadense* L. contributes 8% of the world's cotton, but it is characterized by its superior-quality fibers. The genome of tetraploid cotton is complex, and the studies on its genome lag behind other crops. However, cotton is the main resource for natural fiber supply, and new achievements have been obtained with the development of molecular biology.

The advent molecular marker technology makes it possible to construct molecular genetic linkage maps to study cotton genome organization, map QTLs, etc. The first application of DNA markers in cotton genomic research is restriction fragment length polymorphism (RFLP) (Reinisch et al., 1994). Besides, polymerase chain reaction (PCR)-based DNA marker methods have been widely used. Several types of PCR-based DNA markers have been utilized in cotton genome research, such as randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR), and sequence-related amplified polymorphism (SRAP) summarized by Zhang et al. (2008a). Among all the molecular markers, SSRs are more adopted. SSRs are DNA sequences consisting of tandemly repeated arrays of short (1–6 nucleotides) motifs (Field and Wills, 1996). As molecular markers, SSRs combine many desirable marker properties including high levels of polymorphism, codominant amplification pattern, amenability to rapid genotyping assays, and automation.

SSRs can be developed from both coding and noncoding regions of plant genomes (Brown et al., 1996; Scott, 2001). Several resources can be used to search for SSRs including SSR-enriched small insert genomic DNA libraries, bacterial artificial chromosome (BAC) libraries, and expressed sequence tags (ESTs) databases reviewed by Li et al. (2004). In cotton, several projects have been developed to

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isolate SSRs, such as BNL, CIR, CM, DPL, Gh, and JESPR from microsatellite-enriched genomic libraries, MUSB and TMB from BAC-end sequences, and HAU, MGHEs, MUSS/MUCS, NAU and STV from EST database. Currently, a total of approximately 9001 SSRs have been developed in cotton (Blenda et al. 2006; <http://www.cottonmarker.org>).

The characteristics of SSR make it ideal for mapping purpose, and several high-density linkage maps comprised of SSRs have been developed. Rong et al. (2004) constructed a genetic linkage map of the allotetraploid cotton that comprised 2584 loci at 1.74-cM intervals, and BNL-SSRs were mapped in this map. Lacape et al. (2003) and Nguyen et al. (2004) mapped BNL and CIR-SSRs in a 1160-locus map. Park et al. (2005) mapped MUSS/MUCS-SSRs combined with BNL, JESPR, and CIR-SSR; Frelichowski et al. (2006) mapped MUSB-SSRs combined with BNL, JESPR CIR, and NAU-SSR. He et al. (2007) mapped BNL, JESPR, CIR, TMH (after named TMB), MGHEs, CM, and some NAU-SSRs in a linkage map with 1029 loci. Yu et al. (2007) developed a linkage map with 1097 loci and mapped BNL, JESPR, CIR, TMH, MGHEs, MUCS, and some NAU-SSRs. Guo et al. (2007) mapped BNL, JESPR, CIR, TMH, MUSS/MUCS, and NAU-SSRs in a 1790-locus map. Zhang et al. (2008b) mapped BNL, CIR, CM, DPL, HAU, JESPR, MGHEs, MUSB, MUSS/MUCS, TMB, STV and some NAU-SSRs in a 917-locus SSR map. Until now, all the developed SSRs excepted for Gh-SSRs have been mapped into cotton inter- and intraspecific genetic maps. The genetic mapping of SSRs in cotton makes it possible to characterize SSR motifs on cotton chromosomes. In our study, SSR types and motifs were characterized in cotton interspecific genetic maps based on the above motioned linkage maps because much more markers were mapped on these maps.

2 Methods

2.1 Source of mapped SSRs

SSRs mapped in the most informative and high-density interspecific linkage maps of cotton (Nguyen et al., 2004; Rong et al., 2004; Frelichowski et al., 2006; Guo et al., 2007; He et al., 2007; Yu et al., 2007; Zhang et al., 2008b) were retrieved from the original maps and deposited in a Microsoft Excel® file according to the maps and chromosomes.

2.2 Preprocessing of SSR data

Only one copy of SSR locus was retained if it was mapped on the same chromosome by different linkage maps. If one SSR detected duplicated loci on different chromosomes, they were all retained on respective chromosomes. If one SSR detected duplicated loci on the same chromosome and

the loci cosegregated (interval distance between the two loci was 0.0 cM), only one locus was considered; otherwise, they were all remained.

2.3 Assignment of motif to corresponding SSR locus

SSR motifs were assigned to corresponding locus according to the original papers where these SSR markers were first published. If no motifs could be found for one SSR locus, then it was excluded in this study.

2.4 Frequency of SSR type and motif

Frequency of SSR types and motifs were accounted in the Microsoft Excel® file; however, when analyzing SSR motif, base complementarity was taken into account.

3 Results

3.1 SSR loci on the cotton chromosomes

A total of 4800 SSR loci were mapped in the seven informative high-density genetic linkage maps. After trimming the data, only 2902 nonredundant SSR loci with known motif were left (online Supplementary Table S1). Among them, 2216 loci were only mapped in a single map; 336 loci were commonly mapped by two maps; 144 loci were commonly mapped by three maps; 90 loci were commonly mapped by four maps; 68 loci were commonly mapped by five maps; 37 loci were commonly mapped by six maps; and 11 loci were commonly mapped by all seven maps. Among the 26 chromosomes, C02 and C04 had the minimum number of SSR loci; C11 and C19 had the maximum number of SSR loci that was almost three times of that C02 and C04 (Table 1).

3.2 Characterization of different sources of SSRs on individual chromosome

SSRs from different sources were distributed unevenly on cotton chromosomes. For the SSRs derived from enriched libraries, C19 had the most loci, and C18 had the least loci. For the EST-SSRs, C19 held the most loci, and C02 held the least ones. However, the maximum loci of BAC-end SSRs were mapped on C11, and the minimum on C26. C18 was the chromosome that held the most contrast number between enriched library-derived SSRs and EST-SSRs (Fig. 1).

3.3 Overview of SSR type in the whole genome

According to the references, eight types of SSR were identified including 1–6 bp perfect motifs, interrupted and compound SSRs (Jong et al., 2003). Only one mono-nucleotide was found among the 2902 loci; di- and

Table 1 Summary of the genetically mapped SSR loci with known motif on the 26 chromosomes

chromosomes	no. of supported maps							loci/Chr.
	1	2	3	4	5	6	7	
C01	66	12	3	2	1	2	0	86
C02	50	8	2	1	3	0	1	65
C03	85	11	8	2	1	0	0	107
C04	43	14	4	0	2	0	0	63
C05	120	9	13	2	2	2	0	148
C06	80	9	5	2	3	1	0	100
C07	92	3	3	3	1	0	0	102
C08	90	16	7	2	3	1	2	121
C09	95	13	7	3	5	2	1	126
C10	67	15	4	3	2	2	0	93
C11	127	20	6	4	5	8	0	170
C12	82	13	9	5	1	1	0	111
C13	70	13	5	6	2	0	0	96
C14	83	11	3	3	3	2	0	105
C15	101	23	7	2	3	4	0	140
C16	82	9	4	4	0	1	1	101
C17	72	8	5	3	2	0	0	90
C18	96	10	3	4	1	1	5	120
C19	135	15	6	10	7	1	1	175
C20	82	13	3	7	3	1	0	109
C21	106	15	6	2	3	1	0	133
C22	74	12	5	0	1	0	0	92
C23	91	14	5	2	3	1	0	116
C24	87	22	7	3	5	0	0	124
C25	62	11	8	8	4	2	0	95
C26	78	17	6	7	2	4	0	114
total loci	2216	336	144	90	68	37	11	2902

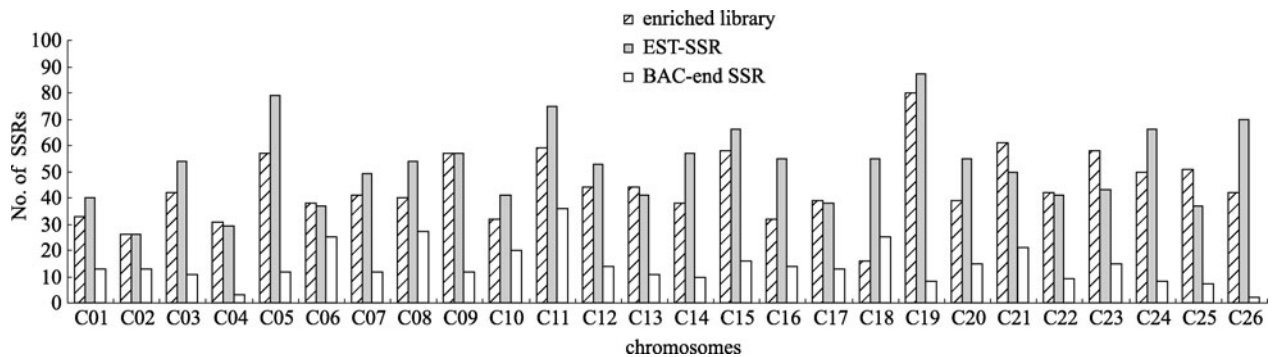


Fig. 1 Distribution of different sources of SSRs on the cotton chromosomes

trinucleotide predominated among all the SSR types (42.07% and 25.40%, respectively), and the others had the similar frequency. When considering the sources of SSRs (only 2–6 bp motifs were considered), enriched library-derived SSRs contributed more than a half of

dinucleotide, less tetranucleotide, and no penta- and hexanucleotide; EST-SSRs contributed all the hexanucleotide, most of the tri-, tetra-, pentanucleotide, and part of the dinucleotide; BAC-end SSRs only contributed a part of 1–5 bp repeat (Fig. 2).

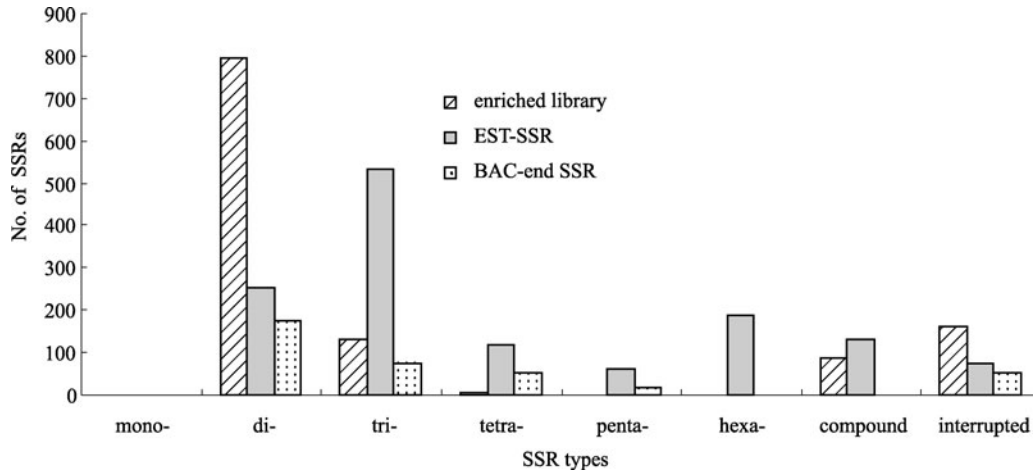


Fig. 2 Number of different SSR types in the cotton genome

3.4 Characterization of SSR type on individual chromosome

Based on all the SSRs data, dinucleotide was the first frequent type on all chromosomes ranging from 30 to 78 loci; trinucleotide was the second frequent type except on C02 ranging from 8 to 45 loci (Table 2). In total, di- and trinucleotide accounted for 53%–79% of the total loci on respective chromosome. Tetranucleotide was frequently mapped on C02, C09, C10, C11, and C15 (> 10), and infrequently mapped on C04, C06, and C13 (< 3). Pentanucleotide was frequently mapped on C11 and C21. Hexanucleotide was frequently mapped on C03, C05, C06, C12, C19, and C24 (> 10) and infrequently mapped on C10, C14, C22, and C25 (< 3).

3.5 Characterization of SSR motifs in the cotton genome

A total of 239 kind of SSR motifs were identified from the 2902 loci (compound and interrupted SSR were excluded) including one mononucleotide (A/T), 5 di-, 29 tri-, 47 tetra-, 39 penta-, and 188 hexanucleotide (online Supplementary Table S2). Although a lot of hexanucleotide motifs were identified, each motif was represented not more than five times.

All the dinucleotide motifs were frequent. Among the trinucleotide motifs, GAA/TTC, AAG/CTT, AAT/ATT, and AGA/TCT were the most frequent ones. ACAT/ATGT, GAAA/TTTC, and CATA/TATG were the most frequent ones in the tetranucleotide motifs. AG/CT most frequently distributed on C05 and C21, CA/TG on C06, GA/TC on C09 and C19, AT/TA on C05, AC/GT on C19, GAA/TTC on C19 and C23 (Table 3).

When an individual chromosome was taken into account, C05, C11, and C19 were the motif-rich chromosomes, while C02 and C04 were the motif-poor chromosomes (Fig. 3). However, C09 and C11 were the motif

type-rich chromosomes, while C04 and C25 were the motif type-poor chromosomes (Fig. 4).

3.6 Characterization of different source of SSR motifs

For the three sources of SSR, dinucleotides and trinucleotides repeat were the most frequent types. Trinucleotides repeat was most abundant in EST-SSRs. For this reason, only dinucleotides and most frequent trinucleotides repeat motifs were characterized in this section. Among the dinucleotides repeat motifs, no AT/TA was found in SSRs from enriched library; on the contrary, it was the most frequent one in EST-SSRs; and few AG/CT and AC/GT were found in BAC-end SSRs. Among the most frequent trinucleotides repeat motifs, GAA/TTC was frequent both in SSRs from enriched library and BAC-end SSRs, and AAG/CTT was also frequent in BAC-end SSRs. However, few CAA/TTG and TGC/GCA were found in SSRs from enriched library, and AGA/TCT, ATC/GAT, and CAA/TTG in BAC-end SSRs; no CAG/CTG was found both in SSRs from enriched library and BAC-end SSRs and TGC/GCA in BAC-end SSRs (Fig. 5).

4 Discussion

In this study, nearly 5000 mapped SSR loci in seven informative, high-density genetic linkage maps were jointly analyzed. After excluding those common loci, 2902 nonredundant SSR loci with known motif were remained. Among the 26 chromosomes, C11 and C19 harbored the most loci, and C02 and C04 harbored the least loci. All repeat types were mapped on the cotton genome, but only one mononucleotide was found among the 2902 loci; di- and trinucleotide were the most frequent ones, which were mainly contributed by enriched library-derived SSRs and EST-SSRs, respectively. Few 4–6 bp repeats

Table 2 Distribution of SSR types on the 26 chromosomes

chromosome	SSR type							
	mono-	di-	tri-	tetra-	penta-	hexa-	complex	compound
C01	–	40	16	4	2	6	7	11
C02	–	31	8	11	2	4	4	5
C03	–	39	18	9	4	17	12	8
C04	–	30	20	0	1	5	3	4
C05	–	69	32	9	1	13	12	12
C06	–	47	25	1	5	11	4	7
C07	–	39	35	5	1	6	10	6
C08	–	50	38	3	2	8	7	13
C09	–	56	30	11	2	10	6	11
C10	–	38	24	12	3	2	6	8
C11	–	53	45	20	9	3	15	25
C12	–	45	33	8	1	11	5	8
C13	–	39	23	1	3	7	12	11
C14	–	37	34	8	5	2	10	9
C15	–	61	26	13	4	4	14	18
C16	–	42	31	8	3	6	2	9
C17	–	40	20	7	4	6	4	9
C18	–	45	39	6	4	8	11	7
C19	–	78	44	6	1	13	10	23
C20	–	47	34	4	4	3	9	8
C21	1	59	29	5	8	7	9	15
C22	–	41	18	6	6	2	5	14
C23	–	59	28	5	2	6	9	7
C24	–	46	30	5	2	15	12	14
C25	–	43	21	3	0	2	8	18
C26	–	47	36	4	1	10	9	7
average	0.04	47.0	28.3	6.7	3.1	7.2	8.3	11.0
total	1	1221	737	174	80	187	215	287

were derived from enriched libraries because enriched library method mainly targeted 2–3 bp repeats (Connell et al., 1998; Reddy et al., 2001; Nguyen et al., 2004). However, the AT/TA motif was mainly derived from EST-SSRs rather than from enriched libraries (Fig. 5). EST-SSRs covered all repeat types except mononucleotide with especially high frequency for the trinucleotide; moreover, hexa-nucleotide was only found in EST-SSRs in present studies. The high frequency of trinucleotide in EST-SSRs was in coincidence with many other crops (Kantety et al., 2002; Tian et al., 2004; Yi et al., 2006). The results indicated that EST-SSRs could efficiently mapped SSRs with different motifs, especially for those with rare motif types.

ESTs were a valuable resource for SSR development, and isolating SSRs from ESTs have become a popular method in many crops because of its high efficiency and low cost. Up to the end of 2008, more than 370000 ESTs are deposited in the Genbank (http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html).

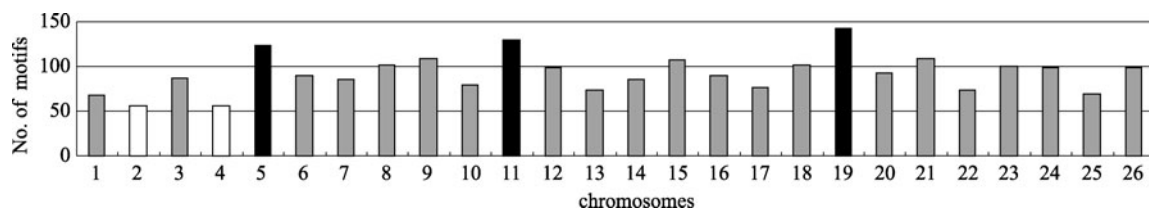
However, only a small part of them have been used to develop SSRs (Qureshi et al., 2004; Park et al., 2005; Taliercio et al., 2006; Han et al., 2004, 2006; Wang et al., 2006; Zhang et al., 2007). The development and mapping of more EST-SSRs would help us get more insights into the structure and architecture of the cotton genome.

There was more dinucleotide in BAC-end SSRs than in enriched library-derived SSRs and EST-SSRs, which was resulted from that only SSR locus with repeat motif of CA, GA, TA, or GAA were identified for TMB markers (Guo et al., 2008). The reason of no mononucleotide and hexanucleotide in BAC-end SSRs was that only 2–5 repeat motifs were identified for MUSB markers (Frelchowski et al., 2006).

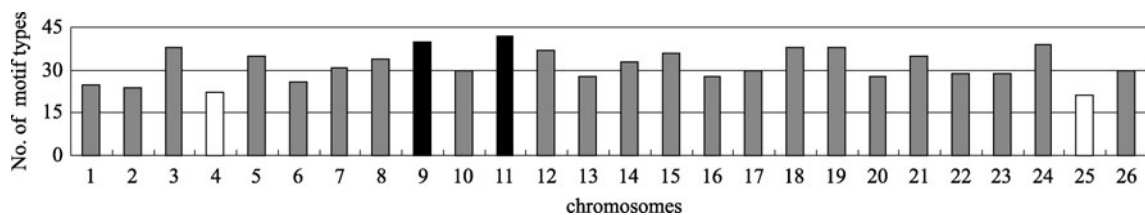
Different SSR types unequally distributed on cotton chromosomes, which would be helpful for adding markers to those chromosomes with less mapped SSR loci. It is feasible to enrich C02 and C10 by adding tetranucleotide

Table 3 The most frequent SSR motifs in cotton genomes

chromosome	motif								
	AG/CT	CA/TG	GA/TC	AT/TA	AC/GT	GAA/TTC	AAG/CTT	AAT/ATT	AGA/TCT
C01	11	5	5	10	9	5	1	0	0
C02	5	6	7	8	5	2	0	0	1
C03	6	10	6	10	7	3	4	0	3
C04	4	7	7	7	5	2	0	3	1
C05	24	9	10	14	12	3	7	0	2
C06	7	21	4	12	3	4	6	5	2
C07	15	11	5	5	3	4	5	1	5
C08	9	16	10	11	4	7	3	5	2
C09	14	13	17	7	5	7	5	1	3
C10	9	7	10	7	5	3	1	5	5
C11	18	7	13	9	6	4	3	3	4
C12	11	9	9	12	4	3	2	1	3
C13	8	15	7	4	5	5	5	1	2
C14	8	15	5	7	2	5	5	6	4
C15	20	8	9	9	15	3	0	1	3
C16	11	4	10	10	7	5	4	1	4
C17	8	15	8	7	2	4	3	2	2
C18	7	13	13	6	6	5	3	5	0
C19	15	18	18	7	20	10	3	6	3
C20	8	8	13	10	8	3	3	5	2
C21	23	12	8	6	10	8	2	2	1
C22	6	9	6	8	12	2	1	1	1
C23	16	14	14	7	8	9	1	0	0
C24	9	10	13	4	10	3	6	1	2
C25	12	12	9	5	5	1	0	5	0
C26	13	9	8	10	7	7	6	0	4
total	297	283	244	212	185	117	79	60	59

**Fig. 3** Number of SSR motifs on the cotton chromosomes

Note: White column showed chromosomes with less motif number; black column showed chromosomes with more motif number.

**Fig. 4** Number of SSR motif types on the cotton chromosomes

Note: White column showed chromosomes with less number of motif types; black column showed chromosomes with more number of motif types.

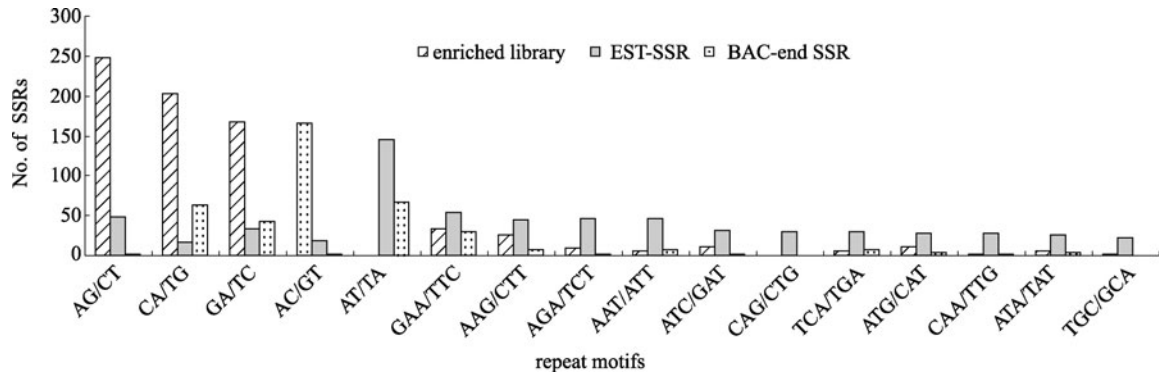


Fig. 5 Dinucleotide and the most frequent trinucleotide repeat motifs in different source of SSRs

SSRs, C03, C06, and C12 by hexanucleotide ones. As for those SSRs mainly derived from ESTs, ESR-SSRs are still a robust way in cotton mapping. However, it appears that it is difficult to enrich C04 because of the low frequency of all SSR types on it.

A total of 239 kind of perfect SSR motifs were identified from the mapped loci; the hexanucleotide had the most motifs but less frequency in each type. Most trinucleotide motifs were identified, but the frequency was different for them; the low-frequency motifs were mostly derived from EST-SSRs. The survey of motifs on cotton chromosomes showed a bias motif distribution (Table 3, Figs. 3 and 4), which maybe help us to further enrich these chromosomes by motif-specific SSRs for map-based cloning and to study microsatellite dynamics on cotton genome.

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