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Short communication

Development of novel EST-SSR markers for cucumber (*Cucumis sativus*) and their transferability to related species

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ABSTRACT

We report on the development of novel simple sequence repeat (SSR) markers from publicly available *Cucumis sativus* expressed sequence tags (ESTs) and on their transferability among related species. In total, 533 di- to penta-type SSRs were identified from 6344 cucumber ESTs retrieved from GenBank. Identified SSRs mainly comprised of di- and tri-nucleotide repeats, of which AG and AAG motifs were much abundant. A total of 392 SSR-containing unigenes (non-redundant ESTs/consensus sequences) were suitable for primer design. From these, 35 primer pairs were designed as representative samples and 28 were usable markers. Twenty-six out of 28 usable markers revealed polymorphism among 21 cucumber accessions with 2–7 alleles detected (mean = 3.77) and their polymorphism information content (PIC) values ranged from 0.091 to 0.748 (mean = 0.388). The polymorphism observed herein partially arose from the null alleles which occurred at the multiple homoeoloci detected by the markers. Transferability of the 28 EST-SSR markers was investigated in four other cucurbits: melon, watermelon, pumpkin and gourd which showed frequency of 92.9%, 57.1%, 53.6% and 60.7%, respectively. The EST-SSR markers developed herein will complement the currently available genomic SSR markers and may be useful for genetic studies in cucumber and related species.

1. Introduction

Microsatellites or simple sequence repeats (SSRs), represented by the repeats of 1–6 nucleotide-long DNA motifs arranged in tandem, have been considered as one of the most powerful Mendelian markers (Jarne and Lagoda, 1996) because of their high reproducibility, co-dominance inheritance, multi-allelic character and extensive genome coverage (Powell et al., 1996). The polymorphism of SSR, mainly resulted from the number variation of repeat units, can easily be detected by simple PCR technique using primers flanking the SSR motifs. These features have advances in genetic mapping, quantitative trait loci (QTL) association, population genetics and evolutionary studies. However, development of SSR markers via traditional methods requires sequence information which often involves a costly and time-consuming procedure of screening of small-insert genomic DNA libraries and subsequent probing with radioactive-labeled probes (Watcharawongpaiboon and Chunwongse, 2008).

Recently, with rapid increase of EST (expression sequence tag) sequences in public database, exploitation of EST-derived SSRs has become more and more feasible by means of bioinformatic tools, such as SSRIT (Temnykh et al., 2001) and MSIA (Thiel et al., 2003). With these tools, SSRs are easily obtained by electronic search of EST databases. To date, an increasing number of EST-SSR markers have been identified and used for multiple applications in a variety of plant species (Varshney et al., 2005; Ellis and Burke, 2007).

In cucumber (*Cucumis sativus*), one of the economically important vegetables in the world, efforts have led to the development of genomic SSR markers (Danin-Poleg et al., 2001; Fazio et al., 2002; Watcharawongpaiboon and Chunwongse, 2008) and their utilization for genome mapping (Danin-Poleg et al., 2000; Fukino et al., 2008) and diversity analysis (Zhuang et al., 2008; Mu et al., 2008). Although over 6000 cucumber ESTs have been deposited in GenBank (http://www.ncbi.nlm.nih.gov/sites/entrez), the utilization of available EST information for SSR marker development has been restricted to few reports (Kong et al., 2006; Yuan et al., 2008). As a consequence, only a total of 48 cucumber EST-SSR primers have been documented which are not only far behind that of the major field crops but also much less than what demanded in cucumber breeding. On the other hand, synteny to other cucurbitaceous species is only based on a limited number of genetic markers, reinforcing the need for more EST-SSR markers for cucumber. Here, we report on the development of novel cucumber EST-SSR markers from GenBank and on their transferability to related species.

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Table 1
Twenty-one cucumber accessions included in present study.

Accession	H/L	Ecotype	Origin
L53	L	NC	China
L57	L	NC	China
L102	L	NC	China
L107	L	NC	China
D01108	L	NC	China
D0462	L	NC	China
Naire	L	NC	China
JY30	H	NC	China
JY10	H	NC	China
L109	L	SC	China
HN007	H	SC	China
HN010	H	SC	China
HN013	H	SC	China
HN016	H	SC	China
HN022	H	SC	China
SHG	L	WS	China
D0351	L	EG	Holland
L112	L	EG	Holland
D0442	L	EG	Holland
DZ-1	L	EG	Holland
D01105	L	AP	USA

H, commercial or experimental hybrid; L, inbred line; NC, North China type; SC, South China type; EC, European greenhouse type; AP, American processing type; WS, wild related species (*Cucumis hystrix* Chakr.).

Table 2
The frequency of the major repeat motifs in cucumber EST sequences.

Type of repeats	Motifs	Number	Frequency (%)
Dinucleotide	AG/CT	176	33.02
	AT/AT	52	9.76
	AC/GT	17	3.19
Trinucleotide	AAG/CTT	109	20.45
	AAC/GTT	13	2.44
	AAT/ATT	14	2.63
	ACC/GGT	16	3.00
	ACG/CTG	12	2.25
	ACT/ATG	21	3.94
	AGG/CCT	12	2.25
	AGC/CGT	11	2.06
	AGT/ATC	16	3.00
Tetranucleotide	AAAG/CTTTT	8	1.50
	AAAC/GTTT	4	0.75
	AAAT/ATTTT	6	1.13
	AATT/AATT	3	0.56
	AATC/AGTT	3	0.56
	AGCT/ATCG	9	1.69
Pentanucleotide	AAAAG/CTTTT	7	1.31
	AAATT/AATTT	3	0.56
	AAAGG/CCTTT	3	0.56

The motifs with a frequency of <0.5% were not listed in the table.

2. Materials and methods

2.1. Plant materials and DNA extraction

To screen polymorphic EST-SSRs, 21 *C. sativus* accessions belonging to various ecotypes were used in present study (Table 1). The four related species, *C. melo* ('C361′ and 'Mapao'), *Citrullus lanatus* ('BC-1′ and 'Heimeiren'), *Cucurbita moschata* ('Juren' and 'Huanglang') and *Lagenaria siceraria* ('Guoguo' and 'Chunye') were used for studying the transferability of cucumber EST-SSRs. All materials were supplied by Group of Plant Genetics and Breeding, Henan Agricultural University, China, and subjected to DNA extraction using the CTAB method described by Murray and Thompson (1980).

2.2. Development of EST-SSR markers

Sequences of 6344 cucumber ESTs were obtained from GenBank, which were assembled using CAP 3 (Huang and Madan, 1999) and resulted in 4036 unigenes (including 832 contigs and 3204 singletons). Application of unigene sequences favours elimination of redundancy and avoidance of possible overestimation (Kong et al., 2006). A web tool, SSRIT (http://www.gramene.org/db/markers/ssrtool), was used for searching SSRs in the unigenes with the criteria as follows: 5 repeats for di- and tri-nucleotide repeats, and 4 repeats for tetra- and penta-nucleotide repeats. With Primer Premier 5.0 program (PREMIER Biosoft International), primers were designed flanking the SSRs and allowed to generate PCR products 100–300 bp in length and annealing temperature (T_a) of 50–60 °C.

2.3. EST-SSR amplification

PCR amplification was performed in a 15-μl system containing 1×PCR buffer, 50 ng of sample DNA, 0.4 μM of each primer, 200 μM of each dNTP, 1.5 mM $MgCl_2$ and 0.5 unit of *Taq* DNA polymerase. All amplifications were carried out in a PTC-200 thermal cycler (MJ Research) as follows: 5 min at 94 °C, followed by 28 cycles of 40 s at 94 °C, 40 s at annealing temperature (T_a) and 1 min at 72 °C, and 8 min at 72 °C for final extension. Amplified products were electrophoresed in 6% denaturing polyacrylamide gels (19:1 acrylamide:bis; 8 M urea) and the gels were silver-stained accord-

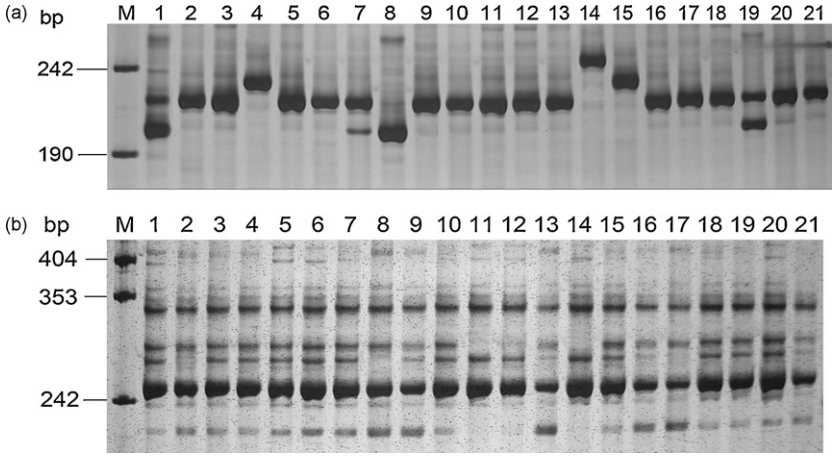

Fig. 1. Band patterns amplified by EST-SSR markers EC49 (a) and EC57 (b). Lane M pUC19 DNA/*Msp*I marker; lane 1, L53; 2, L57; 3, L107; 4, L102; 5, D0462; 6, HN007; 7, D01108; 8, HN013; 9, L109; 10, Naire; 11, HN010; 12, HN016; 13, HN022; 14, D0351; 15, L112; 16, SHG; 17, D0442; 18, JY30; 19, D01105; 20, DZ-1; 21, JY10.