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ORIGINAL PAPER

Development of SSR markers derived from SSR-enriched genomic library of eggplant (*Solanum melongena* L.)

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Abstract Eggplant (*Solanum melongena* L.), also known as aubergine or brinjal, is an important vegetable in many countries. Few useful molecular markers have been reported for eggplant. We constructed simple sequence repeat (SSR)-enriched genomic libraries in order to develop SSR markers, and sequenced more than 14,000 clones. From these sequences, we designed 2,265 primer pairs to flank SSR motifs. We identified 1,054 SSR markers from amplification of 1,399 randomly selected primer pairs. The markers have an average polymorphic information content of 0.27 among eight lines of *S. melongena*. Of the 1,054 SSR markers, 214 segregated in an intraspecific mapping population. We constructed cDNA libraries from several eggplant tissues and obtained 6,144 expressed sequence tag (EST) sequences. From these sequences, we designed 209 primer pairs, 7 of which segregated in the mapping population. On the basis of the segregation data, we constructed a linkage map, and mapped the 236 segregating markers to 14 linkage groups. The linkage map spans a total length of 959.1 cM, with an average marker distance of 4.3 cM. The markers should be a useful resource for qualitative and quantitative trait mapping and for marker-assisted selection in eggplant breeding.

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Introduction

Eggplant (*Solanum melongena* L.), also known as aubergine or brinjal, is a member of the Solanaceae, and is an important vegetable in many countries. Like tomato (*S. lycopersicum*) and pepper (*Capsicum annuum*), eggplant is an autogamous diploid with 12 chromosomes ($2n = 24$). It is a good source of minerals and vitamins and can be compared with tomato in terms of total nutritional value (Kalloo 1993; Singh and Kumar 2007). Despite of its widespread cultivation and economic importance, its molecular genetics have been studied little, in contrast to those of tomato, potato (*S. tuberosum*), and pepper, all of which have high-density linkage maps (Barchi et al. 2007; Jacobs et al. 2004; Tanksley et al. 1992).

Molecular markers have been used extensively to develop genetic and physical genome maps for many basic and applied purposes in crop science, including the Solanaceae (http://www.sgn.cornell.edu/; Doganlar et al. 2002a; Lefebvre et al. 1995; Tanksley et al. 1992). An early eggplant molecular linkage map was based on the tomato restriction-fragment-length polymorphism (RFLP) markers in an interspecific cross between *S.melongena* and *S. linnaeanum* (Doganlar et al. 2002a), and a comparative genetic analysis between eggplant and tomato has been performed (Doganlar et al. 2002a). Recently, conserved ortholog set II (COSII) markers in the Solanaceae have been developed, and a syntenic relationship between eggplant and tomato genomes was analyzed (Wu et al. 2009).

A linkage map of an intraspecific cross of *S. melongena* was based on simple sequence repeat (SSR), randomly amplified polymorphic DNA (RAPD) and amplified-fragment-length polymorphism (AFLP) markers (Nunome et al. 2001, 2003a).

For breeding, it is necessary to detect polymorphisms among cultivars and lines. However, in solanaceous plants, a low frequency of polymorphism among cultivars and intraspecific lines has been reported (Nunome et al. 2003a; Smulders et al. 1997; Stàgel et al. 2008). SSR markers are particularly useful because they are highly variable, easy to use, and amenable to high throughput in most laboratories (Powell et al. 1996; Varshney et al. 2005). A number of SSR markers have been identified in the Solanaceae (Bindler et al. 2007; Minamiyama et al. 2006; Yi et al. 2006), but most of the markers have not been applied to eggplant (Nunome et al. 2003a, b; Stàgel et al. 2008). It is inefficient to apply SSR marker from other species of Solanaceae for analysis of eggplant, despite the close phylogenetic relationship among these species (Broun and Tanksley 1996). Therefore, the development of SSR markers from eggplant would be useful for further development of the eggplant genomic map. We present, here, our progress in the development of eggplant SSR markers derived from SSR-enriched genomic libraries and cDNA libraries with an evaluation of their polymorphism among solanaceous plants.

Materials and methods

Plant materials

A population of 94 F_2 from an intraspecifc cross between EPL1 (a breeding line developed at NIVTS) and WCGR112-8 (introduced from India) were grown in the greenhouse. This population was previously used to construct a linkage map with SSR, AFLP, and RAPD markers (Nunome et al. 2003a). To detect polymorphisms, we used eight lines of *S. melongena,* including EPL1, WCGR112-8, breeding lines, and popular varieties (Table 1). Cross transferability was tested against *S. incanum* LS4021, *S. linnaeanum* LS1151, tomato (*S. lycopersicum*) cv. TPL5 and pepper (*C. annuum*) cv. Mie-midori DNA.

SSR markers

Two SSR-enriched genomic libraries were constructed from genomic DNA of EPL1 by Genomic Identification Services (Chatsworth, CA, USA; A and B in Table 2), and we constructed another two libraries by subtractive hybridization (Nunome et al. 2006; C and D in Table 2). DNA fragments containing SSRs were captured by GA- or CA-repeat oligonucleotides. Clones from each library were picked up randomly and sequenced. The sequence data were base-called, the vector sequence was trimmed, the SSRs were identified, unique clones were selected, and primer pairs were designed. The sequence data were processed with the read2Marker pipeline software (Fukuoka et al. 2005). The SSR markers were labeled with prefixes signifying "eggplant microsatellite": ema, b, d–k.

SSR markers from EST data (EST-SSRs) were also developed. cDNA libraries derived from shoots, roots, immature fruit, and ovaries of EPL1 were constructed using Creator-SMART kit (Invitrogen). More than 6,000 cDNA clones were randomly selected and sequenced from both ends. High quality part of the obtained sequence pair was assembled by phred/phrap and used for the unigene set construction by VISUALBIO-clustering system (NTT Software, Yokohama, Japan) based on FASTA-based global alignment analysis. A total of 3,317 unigenes were identified. SSRs were identified and primer pairs were designed by

Table 1 *Solanum melongena* lines used in this study

Accession[a]		Name	Description	Fruit characters	Origin
JP	NIVTS				
137979	ES123	EPL1	Fusarium wilt resistance	Deep purple, oval-elongated	Mie, Japan
68176	LS3835	WCGR112-8	Bacterial wilt resistance	Green, spherical	India
71134	LS1934	415-121	Bacterial wilt resistance	Green, spherical	Malaysia
–	–	AEP03	Parthenocarpy	Deep purple, oval-elongated	Mie, Japan
138003	ES173	White-egg		White, spherical	Pakistan
33263	LS3809	Nakate-shinkuro	Cultivar	Deep purple, oval-elongated	Japan
33263	LS1730	Senryo 2	Cultivar	Deep purple, oval-elongated	Kyoto, Japan
72038	LS1831	Shironasu	Cultivar	White, oblong	China

JP NIAS genebank, *NIVTS* National Institute of Vegetable and Tea Science

– An accession number has not yet been obtained

read2Marker. The EST-SSR markers were labeled with the prefix ecm. BLASTN analyses were carried out using EST-SSR sequences as queries on http://www.sgn.cornell.edu/.

All of SSR markers were amplified by PCR in 10 μL volumes with 10 ng genomic DNA, 0.25 U *Taq* DNA polymerase (Roche Diagnostics or Takara Bio), 0.2 μM each primer, 200 μM dNTPs, and 1× *Taq* buffer (10 mM Tris–HCl, 1.5 mM $MgCl_2$, 50 mM KCl, pH 8.3) for one cycle of 94°C for 3 min; 10 cycles of 94°C for 0.5 min, 65–55°C decreasing by 1°C per cycle for 1 min, and 72°C for 1 min; 30 cycles of 94°C for 0.5 min, 55°C for 1 min, and 72°C for 1 min; and a final cycle of 72°C for 5 min (PE-9700 thermal cycler, Applied Biosystems). Amplified products were separated by electrophoresis in 2.0% agarose gels with 1× Tris/borate/EDTA (TBE) stained with ethidium bromide, and visualized with UV light. To visualize polymorphisms between *S. melongena* lines, we labeled amplified products with fluorescent dideoxynucleotide according to the method of Kukita and Hayashi (2002). For SSR assays on the mapping population, forward primers were labeled with 6-FAM, VIC, NED, or PET. PCR products were combined with deionized formamide and the GeneScan-500 LIZ internal size standard, and analyzed on a 3730 Genetic Analyzer (Applied Biosystems). Total reactions were visualized with GeneScan 3.7 software and manually scored.

Linkage map construction

Linkage analysis was performed using MAPMAKER/EXP 3.0 (Lander et al. 1987). Markers were grouped with the "group" command at LOD > 4.0. Markers within groups were ordered using the "order" command at LOD > 4.0 and were considered as the frame for each linkage group (LG). Marker distances were calculated with the Kosambi function (Kosambi 1944). The linkage map was drawn with MapChart v. 2.1 (Voorrips 2002).

Data analysis

Polymorphic information content (PIC) values were calculated from fingerprint data from the eight lines as $1 - \Sigma pi^2$, where *pi* is the frequency of an individual genotype, if we assume that each SSR reveals 1 locus (Nei 1987).

Results and discussion

Genomic SSRs

Four SSR-enriched libraries were developed. Two of them were developed by Genomic Identification Services and 5,304 clones were sequenced (A and B in Table 2). Of these, 4,333 (81.7%) contained SSR motifs and 456 (8.6%) unique clones were identified. We developed the other two libraries by subtractive hybridization, and 8,767 clones were sequenced (C and D in Table 2). Of these, 3,906 (44.6%) contained 1 or more SSR motif and 2,645 (30.2%) were unique. The GIS libraries had high SSR frequency and redundancy, and the other two had moderate SSR frequency and low redundancy.

Among the 14,071 sequenced clones from all of the SSR-enriched libraries, 3009 were unique, and a total of 2,265 primer pairs were designed by read2Marker to flank the SSR motifs. Based on the sequence data from 3,009 unique clones, 696 contained GA/CT repeats with a maximum repeat length of 43, while, 1,074 contained CA/GT repeats with a maximum repeat length of 51, and 874 contained AT repeats (Table 3). Others had a complex structure with multiple repeats: 117 contained both GA/CT and CA/GT repeats, and 628 had AT repeats in combination with GA/CT (57) or CA/GT (571), indicating high frequency of AT repeats as well (Table 3). Similar observations have been reported in tomato, potato, and pepper (Broun and Tanksley 1996; Milbourne et al. 1998;

Table 2 Efficiency of SSR content and unique clones of SSR-enriched libraries in eggplant

Library	Developed[a]	Marker prefix	Motif	Analyzed clones	SSR-containing clones		Unique SSR clones		
A	GIS	emb, eme	GA/CT	3,168	2,575	(81.3%)[b]	437[b]	(13.8%)[b]	(17.0%)[c]
B	GIS	ema, emd	CA/GT	2,136	1,758	(82.3%)[b]	372[b]	(17.4%)[b]	(21.2%)[c]
A + B				5,304	4,333	(81.7%)[b]	456	(8.6%)[b]	(10.5%)[c]
C	NIVTS	emf, emg, emh	GA/CT	3,637	2,039	(56.1%)[b]	1441[b]	(39.6%)[b]	(70.7%)[c]
D	NIVTS	emi, emj, emk	CA/GT	5,130	1,867	(36.4%)[b]	1412[b]	(27.5%)[b]	(75.6%)[c]
C + D				8,767	3,906	(44.6%)	2645	(30.2%)	(67.7%)[c]
A + B + C + D				1,4071	8,239	(58.6%)[d]	3009[d]	(21.4%)[d]	

[a] *GIS* Genomic Identification Services, USA. *NIVTS* National Institute of Vegetable and Tea Science

[b] Number and percentage in each library

[c] Percentage of SSR-containing clones

[d] Number and percentage in all libraries

Table 3 Attributes of SSR-enriched libraries

Repeat motif	Number of motif	
(GA/CT) ≥ 7	696	(23.1%)
(CA/GT) ≥ 7	1,074	(35.7%)
(AT) ≥ 5	874	(29.1%)
(GA/CT) ≥ 7 and (AT) ≥ 5	57	(1.9%)
(CA/GT) ≥ 7 and (AT) ≥ 5	571	(19.0%)
(GA/CT) ≥ 7 and (CA/GT) ≥ 7	117	(3.9%)
(GA/CT) ≥ 7, (CA/GT) ≥ 7 and (AT) ≥ 5	20	(0.7%)

Minamiyama et al. 2006), indicating that this feature of SSR–complex structure is common in the Solanaceae.

The PCR products of 1,054 (75.3%) of 1,399 randomly selected primer pairs produced a clear band within the predicted size range and were used for further analysis. The remaining primer pairs gave multiple or no PCR products. A total of 598 primer pairs showed polymorphism among eight lines of *S. melongena*, while 456 were monomorphic, resulting in an average of 2.2 alleles per locus over 1,054 primer pairs. Squirrell et al. (2003) have reported that, of the 25 studies across a variety of plant species, on average 17.7% of loci producing PCR products were monomorphic. Our data suggest that the frequency of polymorphism among eggplant cultivars and lines is low.

We also used the primers to amplify templates from tomato, pepper, and eggplant relatives. PCR amplification gave a detectable amplicon in 31% of 78 randomly selected SSR markers in pepper, 55% in tomato, 90% in *S. linnaeanum*, and 94% in *S. incanum* (Supplementary Table S1, unpublished data): 44% were specific for eggplant (*Solanum*) relatives. The success of amplification appeared to be related to the phylogenetic distance between eggplant and each of the other species (Pearce and Lester 1979). Similarly, the proportion of SSR markers developed for tomato that successfully amplified products in related species decreased with an increasing evolutionary distance (Frary et al. 2005).

EST-SSRs

We constructed EST libraries from several eggplant tissues, because limited sequences were available in the public database when we surveyed it. We obtained sequence data of 6144 ESTs. The non-redundant pool contained 3,317 sequences, of which 209 (3.4%) contained 1 or more SSRs. SSR frequency showed no significant difference from other plants, e.g. pepper (10.2%; Yi et al. 2006), rice (1.6%; Temnykh et al. 2000), grape (2.5%; Scott et al. 2000), and barley (7.5%; Thiel et al. 2003). Similar results have been reported for eggplant (3.4%) (Stàgel et al. 2008). Of the 209 sequences, 92 contained trinucleotide repeats, 30 contained GA/CT repeats, 9 contained CA/GT repeats, and 19 contained AT repeats and the rest had a complex structure with multiple repeats. On BLAST analysis, 19.8% of clones showed significant homology to eggplant EST sequences in the SOL Genomic Network (SGN) database, and 82.3% showed homology to *Solanum* unigenes (Table 4). Significant homology was detected between ecm025 and EEMS39 marker reported by Stàgel et al. (2008), but the marker showed no polymorphism between parental lines of the mapping population and could not be assigned into LG (Table 4).

We designed 96 primer pairs from sequences flanking selected ESTs, of which 76 contained dinucleotide repeats, 45 contained trinucleotide repeats, and 25 contained both. The PCR products of 66 (68.8%) of the primer pairs produced a clear band within the predicted size range and were used for further analysis and the rest amplified no PCR products, for various possible reasons, including the position of primers across a splicing site or a chimeric origin of cDNA clones. A total of 20 primer pairs were amplified and revealed polymorphism among eight lines of *S. melongena*, with an average of 1.4 alleles per locus. The remaining 46 (47.9%) primer pairs were monomorphic. The frequency of monomorphism of EST-SSRs is higher than that of genomic SSRs.

Genomic SSRs versus EST-SSRs

For the genomic SSRs, of the 1,054 primer pairs producing clear products, 598 (56.7%) were polymorphic while 456 (43.3%) were monomorphic. For the EST-SSRs, of the 66 primer pairs producing clear products, 20 (30.3%) were polymorphic and 46 (69.7%) were monomorphic. The genomic SSRs were more polymorphic than the EST-SSRs, with a higher mean number of alleles (2.2 vs. 1.4) and with a higher mean PIC value (0.27 vs. 0.13). In many species, noncoding SSRs are generally more polymorphic than genic SSRs (Cho et al. 2000; Eujayl et al. 2002; Ohyama et al. 2009). Genomic SSRs are useful for genetic analysis using cultivars or closely related lines. Noncoding SSRs were clustered in the centromeric heterochromatin regions (Areshchenkova and Ganal 2002; Brandes et al. 1997; Frary et al. 2005; Ohyama et al. 2009). In constrast, most ESTs are found in euchromatin.

Linkage map

A total of 245 SSR markers showed polymorphism between parents of the mapping population: 222 genomic SSRs, 7 EST-SSRs and 16 SSRs reported by Nunome et al. (2003a,b). We genotyped an intraspecific F_2 population

Table 4 Homology description of EST-SSR markers

Marker	LG	Homologous ESTs in *S. melongena* SGN ESTs ID[a]	e-Value	Homologous SGN unigene ID	e-Value	Annotation
ecm001	3	No hits found		U274702	2.00e-44	VAMP (vesicle-associated membrane protein)-associated protein family
ecm002	–	No hits found		U321159	0	Zinc transporter
ecm003	–	No hits found		U449022	e-124	
ecm004	–	No hits found		No hits found		
ecm005	–	No hits found		No hits found		
ecm006	–	No hits found		No hits found		
ecm007	–	No hits found		U274253	0	Ribosomal protein L29p family
				U428223	0	Ribosomal protein L29p family
ecm008	–	No hits found		U272728	e-144	Glutaredoxin-like protein
ecm009	8	E517118	8.00e-31	U289451	e-110	Pectin methylesterase
ecm010	–	E515352	0	U205601	0	
		E515880	0			
		E514074	0			
		E517260	0			
		E514073	0			
		E517108	0			
		E516004	0			
		E516511	0			
		E514922	0			
		E515146	0			
		E517259	0			
ecm011	–	No hits found		No hits found		
ecm012	–	No hits found		U314450	e-168	Dimethylmenaquinone methyltransferase family
ecm013	–	E519239	0	U271714	0	Multimeric flavodoxin WrbA
				U197160	0	Osmotin-like protein precursor
				U206519	0	Osmotin-like protein precursor
				U318558	0	Osmotin-like protein precursor
ecm014	–	No hits found		U297539	5.00e-59	Beta-hydroxyacyl-(acyl-carrier-protein) dehydratase FabZ
ecm015	–	No hits found		U208120	e-110	60S ribosomal protein L35
ecm016	–	No hits found		No hits found		
ecm017	–	No hits found		No hits found		
ecm018	–	No hits found		U197210	e-166	
ecm019	–	E516017	0	U206044	0	Eukaryotic translation initiation factor 5A-2
		E515769	0	U268312	0	Fetal Alzheimer antigen isoform 2
				U313079	0	Eukaryotic translation initiation factor 5A-2
				U313078	0	Eukaryotic translation initiation factor 5A-2
				U431085	0	Eukaryotic translation initiation factor 5A-1
				U196075	0	Eukaryotic translation initiation factor 5A-2
ecm020	–	No hits found		U275889	0	
				U318972	0	
				U421353	0	Zinc finger (DNL type) family protein
ecm021	–	No hits found		No hits found		
ecm022	–	E515444	0	U205903	0	Ribosomal protein S19
				U270064	0	Ribosomal protein S19
				U334384	0	Ribosomal protein S19
				U313464	0	Ribosomal protein S19

Table 4 continued

Marker	LG	Homologous ESTs in *S. melongena* SGN ESTs ID[a]	e-Value	Homologous SGN unigene ID	e-Value	Annotation
				U196504	0	Ribosomal protein S19
ecm023	7	No hits found		U273313	e-106	Alternative oxidase 1a
ecm024	–	No hits found		U270351	0	
				U312858	0	Putative Rieske Fe–S protein precursor
				U196559	0	Cytochrome B6-F complex iron–sulfur subunit 2
				U447067	0	Cytochrome B6-F complex iron–sulfur subunit
				U445095	0	Cytochrome B6-F complex iron–sulfur subunit
				U507254	0	Cytochrome B6-F complex iron–sulfur subunit
ecm025	–	E518218	0	U272099	0	Kidney epithelial small glutamine rich tricopeptide-containing protein alpha
				U318048	0	Poly(A)-binding protein II
				U206432	0	
				U432235	0	Polyadenylate-binding protein
				U506445	0	Polyadenylate-binding protein
				U206514	e-167	Polyadenylate-binding protein
ecm026	–	E519656	0	U205708	0	Superoxide dismutase
		E515783	0	U268852	0	Superoxide dismutase
		E519657	0	U314405	0	Superoxide dismutase
				U314403	0	Superoxide dismutase
				U196378	0	Superoxide dismutase
				U439960	0	Superoxide dismutase
				U516917	0	Superoxide dismutase
				U439959	0	Superoxide dismutase
ecm027	–	E518602	0	U268651	0	Spermidine synthase
		E518621	0	U314881	0	Spermidine synthase
		E518620	0	U440370	0	Spermidine synthase
				U440368	0	Spermidine synthase
				U415196	0	Spermidine synthase
				U196858	0	Spermidine synthase
				U520134	0	Spermidine synthase 1
				U207727	0	Spermidine synthase
				U205821	0	Spermidine synthase 2
				U440369	0	Spermidine synthase 1
				U314886	0	Spermidine synthase 1
				U206355	0	Spermidine synthase
				U268652	0	Spermidine synthase 1
ecm028	–	No hits found		U313906	e-125	
ecm029	–	No hits found		No hits found		
ecm030	–	No hits found		U324412	e-110	Cyclin family protein
ecm031	2	No hits found		U323006	e-117	Hydroxyproline-rich glycoprotein family protein
				U283746	e-117	
ecm032	9	No hits found		No hits found		
ecm033	–	No hits found		No hits found		
ecm034	–	No hits found		No hits found		
ecm035	–	No hits found		U326284	0	Putative Rar1 protein
				U278581	0	Putative Rar1 protein
ecm036	–	No hits found		U270025	e-150	Copper chaperone

Table 4 continued

Marker	LG	Homologous ESTs in *S. melongena* SGN ESTs ID[a]	e-Value	Homologous SGN unigene ID	e-Value	Annotation
ecm037	–	No hits found		U324351	0	Polysaccharide lyase family 1
				U423123	0	Pectate lyase family protein
ecm038	–	No hits found		U273841	e-104	
ecm039	–	No hits found		No hits found		
ecm040	–	E517891	5.00e-66	U319920	2.00e-86	
ecm041	–	No hits found		U332187	2.00e-48	
ecm042	–	E519141	e-139	U206647	e-137	
ecm043	–	E517619	e-168	U207165	e-166	Putative transcription factor APFI
ecm044	–	No hits found		U278677	e-143	Ras family GTP-binding protein
ecm045	–	No hits found		U324685	0	Protein kinase
ecm046	–	No hits found		No hits found		
ecm047	–	No hits found		U317356	e-121	RNA recognition motif (RRM)-containing protein
ecm048	–	No hits found		U285295	3.00e-79	Heavy-metal-associated domain-containing protein
ecm049	–	E517785	0	U205807	0	Small nuclear ribonucleo protein E
		E517786	0			
ecm050	–	No hits found		U330356	1.00e-55	18S pre-ribosomal assembly protein gar2
ecm051	–	E517176	e-130	U206003	e-127	ATPase beta subunit
ecm052	–	No hits found		U284998	4.00e-42	Mature anther-specific protein LAT61
ecm053	–	No hits found		U326500	e-114	Ornithine carbamoyltransferase precursor
ecm054	–	No hits found		U271648	3.00e-59	
ecm055	–	E519377	0	U206130	0	
		E519411	0			
ecm056	–	No hits found		U298301	1.00e-53	
ecm057	–	No hits found		U291268	1.00e-72	
ecm058	–	No hits found		U322956	e-179	Micro-fibrillar-associated
ecm059	–	No hits found		U323478	e-104	Aminoacylase
ecm060	–	No hits found		U268337	0	Flavodoxin
ecm061	–	No hits found		U326669	0	GRF1-interacting factor 1
ecm062	–	No hits found		U324306	e-101	
				U273658	e-101	Myb family transcription factor
ecm063	–	No hits found		No hits found		
ecm064	–	No hits found		U274114	e-168	B2 protein
ecm065	–	No hits found		U430721	2.00e-78	Hexokinase 6
ecm066	–	No hits found		U274480	0	Serine/threonine protein kinase
				U329612	0	Osmotic stress-activated protein kinase
ecm067	–	No hits found		U272363	0	NADH-ubiquinone oxidoreductase
				U316563	0	NADH dehydrogenase
				U422927	0	NADH dehydrogenase
				U209530	0	NADH2 dehydrogenase
				U422926	0	NADH dehydrogenase
ecm068	–	No hits found		U313100	4.00e-94	Leucine-rich repeat extensin family
ecm069	–	E519420	0	U268665	0	60S ribosomal protein L19
				U313255	0	60S ribosomal protein L19
				U205645	0	60S ribosomal protein L19
				U196098	0	60S ribosomal protein L19
				U444567	0	60S ribosomal protein L19
				U511218	0	60S ribosomal protein L19

Table 4 continued

Marker	LG	Homologous ESTs in *S. melongena* SGN ESTs ID[a]	e-Value	Homologous SGN unigene ID	e-Value	Annotation
ecm070	5	No hits found		U321009	e-117	
ecm071	–	No hits found		U338997	6.00e-36	
ecm072	–	No hits found		No hits found		
ecm073	–	No hits found		U273146	8.00e-51	70 kDa heat shock cognate protein 3
ecm074	–	No hits found		U279158	3.00e-72	Fas (TNFRSF6) associated factor 1
ecm075	–	No hits found		U275836	1.00e-83	Putative auxin response factor 14
ecm076	–	No hits found		U293743	8.00e-43	
ecm077	–	No hits found		U285110	1.00e-50	Gag-pol polyprotein
ecm078	–	No hits found		U271986	2.00e-70	Reticulon 4
ecm079	–	No hits found		U328977	9.00e-47	
ecm080	–	No hits found		U277829	e-104	Acetyl-CoA:benzylalcohol acetyltranferase-like protein
ecm081	–	No hits found		U321904	e-138	Imidazoleglycerol-phosphate dehydratase
				U274817	e-138	Sucrose synthase
ecm082	–	No hits found		U273640	0	Sgn1p-like protein
				U313462	0	Glyoxalase/bleomycin
ecm083	–	No hits found		U274605	2.00e-80	
ecm084	–	No hits found		No hits found		
ecm085	–	No hits found		U344444	8.00e-56	
ecm086	–	No hits found		U318317	0	Acyl-ACP
				U196627	0	Oleoyl-[acyl-carrier-protein] hydrolase
				U451566	0	Acyl-(acyl-carrier protein) thioesterase
ecm087	–	E520208	9.00e-90	U268765	e-143	60S ribosomal protein L35
ecm088	–	E517294	e-120	U313022	e-133	Putative calmodulin
ecm089	–	No hits found		U425324	1.00e-72	
ecm090	3	E514683	0	U269396	0	Ketol-acid reductoisomerase
		E519909	0	U313866	0	
				U356322	0	
				U423213	0	
				U208065	0	
				U205802	0	
				U196833	0	
				U196832	0	
ecm091	–	No hits found		U273054	1.00e-87	Auxin-repressed protein-like protein
ecm092	–	No hits found		U329953	7.00e-47	
ecm093	–	No hits found		U312980	0	Putative sphingolipid delta 4 desaturase DES-1
				U286116	0	Putative sphingolipid delta 4 desaturase DES-1
				U447812	0	
ecm094	–	E514463	0	U269254	0	
				U315928	0	Pyrrolidone carboxyl peptidase-like protein
				U423021	0	
				U514612	0	Pyrrolidone-carboxylate peptidase family protein
				U206909	0	Pyrrolidone carboxyl peptidase-like protein
				U455662	0	
ecm095	–	No hits found		No hits found		
ecm096	–	No hits found		U268882	0	
				U313598	0	

SGN SOL Genomics Network (http://www.sgn.cornell.edu/)

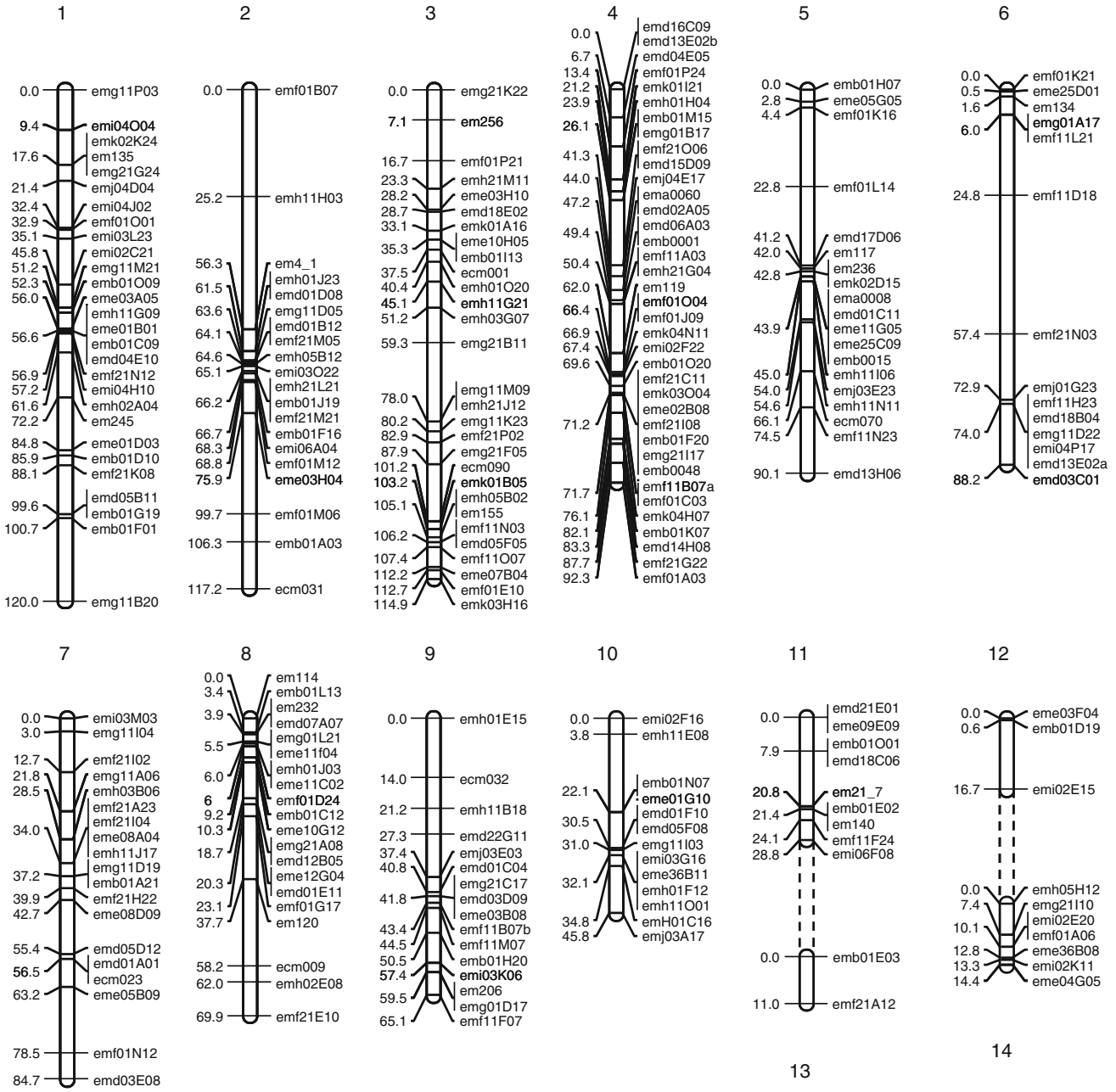

Fig. 1 SSR-based linkage map of eggplant. The linkage map consists of 236 SSR markers and spans a total genetic distance of 959.1 cM in 14 linkage groups. SSR markers that were developed using SSR-enriched libraries are labeled with prefixes ema-k. SSR markers that were developed from EST data are denoted by "ecm". Previously reported SSR markers are denoted by "em". The *dashed line* indicated that the linkage group LG13 and LG14 were linked with LG11 and LG12 by preliminary analysis, respectively

with these 245 SSR markers. The linkage map was constructed with 236 markers: 214 genomic SSRs, 7 EST-SSRs, and 15 published-SSRs (Fig. 1, Supplementary Table S1). The remaining nine markers were assigned into LGs, but could not be mapped. The map spanned a total genetic distance of 959.1 cM in 14 LGs. Preliminary analysis based on the segregation data of SSR markers and previously reported RAPD and AFLP markers (Nunome et al. 2003a) indicated that LGs 13 and 14 were linked with LGs 11 and 12, respectively (Fig. 1), but the distance between them was not clear. LGs ranged in size from 11 cM (LG13) to 120 cM (LG1) and contained between 2 and 37 markers. Distances between markers varied from 0 to 32.6 cM, with an average of 4.3 cM. The linkage map covered 64.8% of the RFLP-based linkage map which consists of 12 LGs, spans 1480 cM, and contains 233 markers (Doganlar et al. 2002a). Pronounced clustering of markers was observed on some linkage groups in our map. This is probably due to the

fact that the map was constructed largely from genomic SSRs, which, as previously mentioned, are frequently located in the heterochromatin regions of chromosomes (Fray et al. 2005; Ohyama et al. 2009).

For a comparative analysis of the Solanaceae, we performed a homology search using the sequences adjacent to SSRs that were mapped to LGs against tomato molecular markers in a tomato EST and BAC-end sequence database. Significant homology was detected only in a limited number of both genomic SSR and EST-SSR markers. Some EST-SSR markers showed homology to *Solanum* unigenes, but the position of these unigenes on the LG was not identified. Because homologous markers could not be located on LGs, the relationship of the LGs to tomato chromosomes remains unclear.

Many of the morphological and agronomic traits in eggplant are also shared by tomato and pepper. In most cases, the genetics of these traits have been thoroughly studied in these latter species (Doganlar et al. 2002b). Therefore, it would be effective to map syntenic markers from closely related *Solanum* species on the LGs. A large number of single- or low-copy-number orthologs (referred to as conserved ortholog set, COS and COSII markers) were identified and the universal PCR primers designed can be used to amplify the corresponding orthologs (Fulton et al. 2002; Wu et al. 2006; Wu et al. 2009) and to make a tighter association between LGs and individual chromosomes. We expect that the integration of these markers to our linkage map will be useful for the comparative analysis of the valuable traits.

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References

Areshchenkova T, Ganal MW (2002) Comparative analysis of polymorphism and chromosomal location of tomato microsatellite markers isolated from different sources. Theor Appl Genet 104:229–235

Barchi L, Bonnet J, Boudet C, Signoret P, Nagy I, Lanteri S, Palloix A, Lefebvre V (2007) A high-resolution intraspecific linkage map of pepper (*Capsicum Annuum* L.) and selection of reduced RIL subsets for fast mapping. Genome 50:51–60

Bindler G, van der Hoeven R, Gunduz I, Plieske J, Ganal M, Rossi L, Gadani F, Donini P (2007) A microsatellite marker based linkage map of tobacco. Theor Appl Genet 114:341–349

Brandes A, Thompson H, Dean C, Heslop-Harrison JS (1997) Multiple repetitive DNA sequences in the paracentromeric regions of *Arabidopsis thaliana* L. Chromosome Res 5:238–246

Broun P, Tanksley SD (1996) Characterization and genetic mapping of simple repeat sequences in the tomato genome. Mol Gen Genet 250:39–49

Cho YG, Ishii T, Temnykh S, Chen X, Lipovich L, McCouch SR, Park WD, Ayres N, Cartinhour S (2000) Diversity of microsatellites derived from genomic libraries and GenBank sequences in rice (*Oryza sativa* L.). Theor Appl Genet 100:713–722

Doganlar S, Frary A, Daunay MC, Lester RN, Tanksley SD (2002a) A comparative genetic linkage map of eggplant (*Solanum melongena*) and its implications for genome evolution in the Solanaceae. Genetics 161:1697–1711

Doganlar S, Frary A, Daunay MC, Lester RN, Tanksley SD (2002b) Conservation of gene function in the solanaceae as revealed by comparative mapping of domestication traits in eggplant. Genetics 161:1713–1726

Eujayl I, Sorrells ME, Baum M, Wolters P, Powell W (2002) Isolation of EST-derived microsatellite markers for genotyping the A and B genomes of wheat. Theor Appl Genet 104:399–407

Frary A, Xu YM, Liu JP, Mitchell S, Tedeschi E, Tanksley S (2005) Development of a set of PCR-based anchor markers encompassing the tomato genome and evaluation of their usefulness for genetics and breeding experiments. Theor Appl Genet 111:291–312

Fukuoka H, Nunome T, Minamiyama Y, Kono I, Namiki N, Kojima A (2005) read2Marker: a data processing tool for microsatellite marker development from a large data set. Biotechniques 39:472–474

Fulton TM, Van der Hoeven R, Eannetta NT, Tanksley SD (2002) Identification, analysis, and utilization of conserved ortholog set markers for comparative genomics in higher plants. Plant Cell 14:1457–1467

Jacobs JME, Van Eck HJ, Arens P, Verkerk-Bakker B, Lintel B, Hekkert HJM, Bastiaanssen A, El-Kharbotly A, Pereira E, Jacobsen E, Stiekema WJ (2004) A genetic map of potato (*Solanum tuberosum*) integrating molecular markers, including transposons, and classical markers. Theor Appl Genet 91:289–300

Kalloo G (1993) Eggplant *Solanum melongena* L. In: Kalloo G, Bergh BO (eds) Genetic improvement of vegetable crops. Pergamon Press, Oxford, pp 587–604

Kosambi D (1944) The estimation of map distance from recombination values. Ann Eugen 12:172–175

Kukita Y, Hayashi K (2002) Multicolor post-PCR labeling of DNA fragments with fluorescent ddNTPs. Biotechniques 33:502–506

Lander E, Abrahamson J, Barlow A, Daly M, Lincoln S, Newburg L, Green P (1987) Mapmaker: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Lefebvre V, Palloix A, Caranta C, Pochard E (1995) Construction of an intraspecific integrated linkage map of pepper using molecular markers and doubled haploid progenies. Genome 38:112–121

Milbourne D, Meyer RC, Collins AJ, Ramsay LD, Gebhardt C, Waugh R (1998) Isolation, characterisation and mapping of simple sequence repeat loci in potato. Mol Gen Genet 259:233–245

Minamiyama Y, Tsuro M, Hirai M (2006) An SSR-based linkage map of *Capsicum annuum*. Mol Breed 18:157–169

Nei M (1987) Genetic variation within species. In: Nei M (ed) Molecular evolutionary genetics. Columbia University Press, NY, pp 176–207

Nunome T, Ishiguro K, Yoshida T, Hirai M (2001) Mapping of fruit shape and color development traits in eggplant (*Solanum melongena* L.) based on RAPD and AFLP markers. Breed Sci 51:19–26

Nunome T, Suwabe K, Iketani H, Hirai M (2003a) Identification and characterization of microsatellites in eggplant. Plant Breed 122:256–262

Nunome T, Suwabe K, Ohyama A, Fukuoka H (2003b) Characterization of trinucleotide microsatellites in eggplant. Breed Sci 53:77–83

Nunome T, Negoro S, Miyatake K, Yamaguchi H, Fukuoka H (2006) A protocol for the construction of microsatellite enriched genomic library. Plant Mol Biol Rep 24:305–312

Ohyama A, Asamizu E, Negoro S, Miyatake K, Yamaguchi H, Tabata S, Fukuoka H (2009) Characterization of tomato SSR markers developed using BAC-end and cDNA sequences from genome databases. Mol Breed (in press)

Pearce K, Lester RN (1979) Chemotaxonomy of the cultivated eggplant: a new look at the taxonomic relationships of *Solanum melongena* L. In: Hawkes JG, Lester RN, Skelding AD (eds) The Biology and Taxonomy of the Solanaceae. Academic Press, London, pp 605–614

Powell W, Machray GC, Provan J (1996) Polymorphism revealed by simple sequence repeats. Trends Plant Sci 1:215–222

Scott KD, Eggler P, Seaton G, Rossetto M, Ablett EM, Lee LS, Henry RJ (2000) Analysis of SSRs derived from grape ESTs. Theor Appl Genet 100:723–726

Singh M, Kumar R (2007) Eggplant (*Solanum melongena* L.). In: Singh RJ (ed) Genetic resources, chromosome engineering, and crop improvement. CRC Press, Boca Raton, FL, USA, pp 473–495

Smulders MJM, Bredemeijer G, Rus-Kortekaas W, Aren P (1997) Use of short microsatellites from database sequences to generate polymorphisms among *Lycopersicon esculentum* cultivars and accessions of other *Lycopersicon* species. Theor Appl Genet 97:264–272

Squirrell J, Hollingsworth M, Woodhead M, Russell J, Lowe J, Gibby M, Powell W (2003) How much effort is required to isolate nuclear microsatellites from plants? Mol Ecol 12:1339–1348

Stàgel A, Portis E, Toppino L, Rotino GL, Lanteri S (2008) Gene-based microsatellite development for mapping and phylogeny studies in eggplant. BMC Genomics 9:357–370

Tanksley SD, Ganal MW, Prince JP, de Vicente MC, Bonierbale MW, Broun P, Fulton TM, Giovannoni JJ, Grandillo S, Martin GB, Messeguer R, Miller JC, Miller L, Paterson AH, Pineda O, Roder MS, Wing RA, Wu W, Young ND (1992) High density molecular linkage maps of the tomato and potato genomes. Genetics 132:1141–1160

Temnykh S, Park WD, Ayres N, Cartinhour S, Hauck N, Lipovich L, Cho YG, Ishii T, McCouch SR (2000) Mapping and genome organization of microsatellite sequences in rice (*Oryza sativa* L.). Theor Appl Genet 100:697–712

Thiel T, Michalek W, Varshney RK, Graner A (2003) Exploiting EST databases for the development and characterization of gene-derived SSR-markers in barley (*Hordeum vulgare* L.). Theor Appl Genet 106:411–422

Varshney RK, Graner A, Sorrells ME (2005) Genetic microsatellite markers in plants: features and applications. Trends Biotechnol 23:48–55

Voorrips RE (2002) MapChart: software for the graphical presentation of linkage maps and QTLs. J Hered 93:77–78

Wu FN, Mueller LA, Crouzillat D, Petiard V, Tanksley SD (2006) Combining bioinformatics and phylogenetics to identify large sets of single-copy orthologous genes (COSII) for comparative, evolutionary and systematic studies: a test case in the euasterid plant clade. Genetics 174:1407–1420

Wu F, Eannetta NT, Xu Y, Tanksley SD (2009) A detailed synteny map of the eggplant genome based on conserved ortholog set II (COSII) markers. Theor Appl Genet 118(5):927–935

Yi GB, Lee JM, Lee S, Choi D, Kim BD (2006) Exploitation of pepper EST-SSRs and an SSR-based linkage map. Theor Appl Genet 114:113–130