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ORIGINAL PAPER

Construction of SSR-based chromosome map in bunching onion (*Allium fistulosum*)

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Abstract We have constructed a linkage map of bunching onion (*Allium fistulosum* L., $2n = 16$) using an F_2 population of 225 plants. The map consists of 17 linkage groups with 212 bunching onion SSR markers and 42 bulb onion (*A. cepa* L.) SSR, InDel, CAPS or dCAPS markers, covering 2,069 cM. This is the first report of a linkage map mainly based on SSR markers in the genus *Allium*. With the 103 anchor markers [81 bunching onion SSRs, 11 bulb onion SSRs and 11 bulb onion non-SSRs (1 InDel, 9 CAPSs and 1 dCAPS)] whose chromosome assignments were identified in *A. cepa* and/or *A. fistulosum*, via the use of several kinds of *Allium* alien addition lines, 16 of the 17 linkage groups were connected to the 8 basic chromosomes of *A. cepa*.

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Introduction

Bunching onion (*Allium fistulosum* L.), also called Japanese bunching onion or Welsh onion, is thought to originate from northwestern China, and is mainly cultivated in East Asian countries, particularly in Japan, China and Korea (Kumazawa and Katsumata 1965; Ford-Lloyd and Armstrong 1993). In Japan, bunching onion accounts for the fourth highest annual output of vegetables, following tomato, strawberry and cucumber (MAFF 2005).

The main breeding objectives for bunching onion are disease resistance, high yield, late bolting, high qualities

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(e.g., low pungency, high sugar content) and suitability for mechanized farming (vigorous seedling growth, etc.). These traits are also important in bulb onion (*A. cepa* L.) breeding. Quantitative trait loci (QTL) analysis based on a genetic linkage map would be efficient for revealing the mode of inheritance of these traits. However, despite the economic importance of bunching onion and bulb onion, its genetic characteristics are poorly studied and molecular-based approaches need to be utilized to clarify such characteristics. By revealing these genetic characteristics, it will be possible to utilize comparative genomics approaches between bunching onion and bulb onion, and molecular breeding for both crops will be conducted.

Simple sequence repeat (SSR) markers are ideal DNA markers owing to their simplicity, reproducibility and codominant inheritance (Jones et al. 1997). In the genus *Allium*, Fischer and Bachmann (2000) first reported the development of SSR markers from a bulb onion genomic library. From a large-scale sequencing of bulb onion expressed sequence tags (ESTs), Kuhl et al. (2004) detected 336 SSR cores among 313 EST sequences and developed 88 EST-SSR markers. In addition, Martin et al. (2005) located 35 EST-SSRs, 43 single nucleotide polymorphisms (SNPs) and 4 insertion-deletion (InDel) markers on medium-density linkage map in bulb onion. This map was simultaneously anchored to bulb onion chromosomes by use of *A. fistulosum*- *A. cepa* monosomic addition lines developed by Shigyo et al. (1996) (Martin et al. 2005). Furthermore, we isolated 1,940 SSR clones from a genomic library and 10 SSR-enriched DNA libraries of bunching onion (Wako et al. 2002a; Song et al. 2004; Tsukazaki et al. 2007).

In the present study, we screened the above-mentioned markers for polymorphisms in a bunching onion F_2 population, developed additional informative markers, and constructed a linkage map of bunching onion mainly based on codominant SSR markers from bunching onion and bulb onion. To assign the detected linkage groups to the 8 basic chromosomes of *A. cepa*, we directly identified the chromosomes to which some anchor markers should belong to using *Allium* alien chromosome addition lines.

Materials and Methods

Plant materials for linkage analysis

A total of 225 individuals of the F_2 progeny derived from a cross between 2 bunching onion inbred lines, D1s-15s-10s and J1s-14s-23s, were used as the mapping population. D1s-15s-10s was an S_3 line from 'Saiko', an open-pollinated cultivar of the Senju group, and J1s-14s-23s was an S_3 line from 'Kujo Futo', an open-pollinated cultivar of the Kujo group. The F_2 mapping population was the selfed progeny of a single F_1 plant between these S_3 lines. Total DNA was extracted from each plant according to the method described by Song et al. (2004).

SSR markers

We screened 266 primer sets previously developed from bunching onion genomic SSR clones (Wako et al. 2002a; Song et al. 2004; Ohara et al. 2005; Tsukazaki et al. 2006, 2007), 1,456 new ones, and 30 from bulb onion (Fischer and Bachmann 2000) for markers showing polymorphisms between the parental lines.

As for bulb onion EST-derived SSR markers, we screened 11,008 bulb onion EST sequences from the Dana-Farber Cancer Institute (DFCI) *A. cepa* Gene Index v.1.0 (http://compbio.dfci.harvard.edu/tgi/cgi-bin/tgi/gimain.pl?gudb=onion) to identify SSRs and design polymerase chain reaction (PCR) primer sequence sets for the detected SSR cores with 'read2Marker' (Fukuoka et al. 2005), and obtained 99 effective primer sequence sets for them. These markers were named as *Allium cepa* EST (ACE) and screened for polymorphisms between the parental lines. We also screened 118 other bulb onion EST-derived SSR markers (ACM) reported by Kuhl et al. (2004) and Martin et al. (2005).

PCR condition was the same as that for the cleaved amplified polymorphic sequence (CAPS) analysis by Ohara et al. (2005). PCR products were separated on 3% (w/v) agarose gel or 5% (w/v) denatured polyacrylamide gel according to the method of Song et al. (2004) or Ohara et al. (2005). In addition, some forward primers were fluorescent-labeled with 6-FAM, NED, PET or VIC dyes (Applied Biosystems, CA, USA) prior to use for PCR. PCR products were loaded on a capillary DNA sequencer (ABI3730; Applied Biosystems), and analyzed using GeneMapper ver. 3.0 software (Applied Biosystems).

InDel and SNP markers

Previously, we developed 39 EST-derived sequence-tagged site (STS) markers of bulb onion named as *Allium cepa* CAPS (ACC) (Wako et al. 2002b). In addition, 208 EST-derived bulb onion STS markers reported by McCallum et al. (2001), Kuhl et al. (2004), and Martin et al. (2005) were used. An STS marker, AOB272 (Gökçe et al. 2002), reported to be closely linked to the bulb onion nuclear male-fertility restoration locus (*Ms*), was also used for our mapping. Moreover, we developed 93 intron spanning EST-derived markers for bunching onion from rice genome sequences. Rice cDNA sequences and their annotation data were obtained from The Institute for Genomic Research

Rice Genome Pseudomolecules ver. 4.0 (ftp://ftp.tigr.org/pub/data/Eukaryotic_Projects/o_sativa/annotation_dbs/pseudomolecules/version_4.0/). We screened the bulb onion EST sequences from DFCI database for those homologous to the rice cDNA sequences (first hit score > 80) considered unique in the rice genome (the ratio of first to second hit *E*-values was higher than 1×10^{10}) by conducting a BLASTN search. The selected bulb onion ESTs were aligned to the rice genome sequences, and primer pairs were designed from highly conserved segments interposing rice intron regions between them so that they could amplify putative intron regions during PCR with the genomic DNA template of either bulb onion or bunching onion.

In total, 341 bulb onion EST-derived marker candidates were screened for InDels and SNPs between the bunching onion parental lines D1s-15s-10s and J1s-14s-23s.

Primer sets producing single amplicons were selected and the fragment sizes were compared between the parental lines on 2% (w/v) agarose gel. When distinct allele differences (>0.1 kbp in fragment size) were identified, the primers were directly applied to F_2 individuals. For identification of SNPs and small InDels, each PCR product was purified using the Wizard CV Gel and PCR clean-up system (Promega, WI, USA), and sequenced with the DNA sequencer ABI3730 (Applied Biosystems). Sequence alignment and SNP/InDel detection were conducted using ATGC ver. 3 software (Genetyx, Tokyo, Japan). The SNPs detected at restriction sites were converted to CAPS markers using GENETYX-WIN ver. 5.1 software (Genetyx). Some other SNPs were converted to the derived cleaved amplified polymorphic sequence (dCAPS) markers using dCAPS Finder 2.0 software (Neff et al. 2002) followed by Primer 3 (Rozen and Skaletsky 2000). When InDels of more than 7 bp were detected, primer sets were redesigned with Primer 3 to amplify smaller fragments with clearer banding differences.

Segregation scoring (or observation) and linkage analyses

Markers showing polymorphisms between the parental lines were applied to F_2 individuals. Some markers did not segregate in a codominant manner probably due to the preferential annealing of primers or small differences in the size of restriction enzyme-digested PCR fragments, and these were treated as dominant markers. Linkage analysis was performed using the computer program MAPMAKER/EXP 3.0b (Lander et al. 1987). The Kosambi function was used to obtain cM values (Kosambi 1944). A log of odds threshold of 6.0 was used to group and order the markers.

Chromosomal assignment of linkage groups

To determine the chromosome identity of the detected bunching onion linkage groups, we used a complete set of bunching onion–shallot (the *A. cepa* Aggregatum group) monosomic addition lines ($2n = 17$; FF + 1C to +8C, where F stands for the basic chromosome set of *A. fistulosum*, and 1C and 8C the first and eighth chromosomes, respectively, of *A. cepa*) developed by Shigyo et al. (1996; note that Shigyo et al. (1996) used the symbol "A" instead of "C" for shallot chromosomes). The chromosomal locations of 30 bunching onion SSRs were directly determined using these monosomic addition lines.

We used the bulb onion chromosome map constructed by Martin et al. (2005) as a reference. Their map consisted of 222 bulb onion markers (RFLPs, SSRs, SNPs and InDels), with the linkage groups assigned to the 8 basic chromosomes of *A. cepa* using Shigyo et al.'s monosomic addition lines described above. We directly compared our bunching onion map with the bulb onion reference map with respect to the 13 bulb onion markers located on both maps. Some other bulb onion-derived markers were assigned to these monosomic addition lines.

On the other hand, we applied 141 markers (139 bunching onion- and 2 bulb onion-derived SSR markers) to hypo-allotriploids in which one of the alien chromosomes are missing from the *A. fistulosum* complement in the diploid background of shallot ($2n = 23$; CCF-1F, CCF-4F, CCF-6F, CCF-7F and CCF-8F, where C and F stand for the basic chromosome sets of *A. cepa* and *A. fistulosum*, respectively, and 1F, for example, stands for the first chromosome of *A. fistulosum*) developed by Hang et al. (2004) and Yaguchi et al. (2008). According to a suggestion in their previous report, these hypo-allotriploids are designated hereafter as single-alien deletions in this report.

Results

SSR markers

Of 1,722 bunching onion genomic SSR primer sets, 1,406 (81.6%) appeared to amplify single loci, and 398 of them detected polymorphisms between the parental lines (Table 1). The efficiency of polymorphism detection was 23.1% by the primer sets designed and 28.3% by the STS markers amplifiable with the primer sets. On the other hand, STS frequency was low (43.3%) for bulb onion genomic SSRs in bunching onion (Table 1). More than 50% of these primers could not amplify bunching onion DNA fragments, or produced multiple bands. In contrast, 75.1% of bulb onion EST-derived SSR markers were clearly amplified in bunching onion. The frequency of polymorphic markers in bulb onion EST-SSRs (11.0%) was comparable to that in bulb onion genomic SSRs (15.4%).

We thus obtained 398 bunching onion SSR markers and 20 bulb onion SSR markers (18 EST-derived and 2 geno-

Table 1 Number of bunching onion- and bulb onion-derived SSR markers used for our bunching onion map

	No. of primer sets designed	STS markers		Polymorphic markers		No. of markers applied to F_2	Marker name	No. of SSR markers mapped
		No.	%	No[a]	%[b]			
Bunching onion								
Genomic SSR	1,722	1,406	81.6	398	28.3	259	AFA, AFAA, AFAT,AFB, AFC, AFD, AFHT, AFRA, AFRT, AFS	212
Bulb onion								
Genomic SSR[c]	30	13	43.3	2	15.4	2	AMS	1
EST-derived SSR (original)	99	78	78.8	12	15.4	12	ACE	11
EST-derived SSR (reported[d])	118	85	72.0	6	7.1	6	ACM	5
EST-derived SSR (total)			75.1		11.0			
Total	1,969	1,582	80.3	418	26.4	279		229

[a] Markers showing polymorphisms between the parental lines, D1s-15s-10s and J1s-14s-23s

[b] No. of polymorphic markers/No. of STS markers obtained (%)

[c] Fischer and Bachmann (2000)

[d] Kuhl et al. (2004) and Martin et al. (2005)

mic SSR markers) showing polymorphisms between the bunching onion parental lines, and applied 259 of the bunching onion SSR markers and all of the bulb onion ones to our F_2 population (Table 1).

InDel and SNP markers

From 11,008 bulb onion EST sequences, 1,090 were selected as highly homologous to rice unigene cDNA sequences. Of them, 19 were already used for developing EST markers for bulb onion by Kuhl et al. (2004) and Martin et al. (2005). From the remaining 1,071 sequences, we designed 93 new primer sets for intron spanning EST markers for bunching onion (Table 2). These 93 original marker candidates, together with 248 previously reported ones, were examined for amplifiability in bunching onion.

From a total of 341 primer sets, 253 (74.2%) appeared to amplify single loci in bunching onion (Table 2). So far, we have sequenced 195 of the amplified loci in each parent and found 3 informative markers (ACAAJ79, ACAAX07 and TC1952) with large (>0.1 kbp) InDels between the parents (Fig. 1a). These InDels were detected in intron regions (Fig. 1b).

Sequence comparison of the 192 other loci between the parental lines detected 55 polymorphic loci: 13 small InDels (1–11 bp) at 10 loci and 134 SNPs at 54 loci, with 9 loci containing both types of polymorphisms. By including the large InDels described above, the frequency of polymorphic markers was 29.7% [(3 + 55)/195 loci]. As for the SNPs detected, most (95 SNPs, 70.9%) of them were due to transition. The frequency of SNP detection was estimated at 1.5 per 1 kbp sequence.

Table 2 Numbers of bulb onion EST-derived InDel, CAPS and dCAPS markers used for our bunching onion map

Primer sequence source	No. of primer sets examined	STS markers			No. of markers sequenced	InDel/SNP markers[a]			No. of InDels detected	No. of SNPs detetcted	No. of markers applied to F_2	Marker name	No. of non-SSR markers mapped	
		No.	%	No.		%	InDel	CAPS					dCAPS	Total
Original	93	71	76.3	52	13	25.0	4	20	5	Misc.[b]	2	2	1	4
Reported[c]	39	27	69.2	20	8	40.0	1	33	7	ACC		7		7
Reported[d]	209	155	74.2	123	37	30.1	11	81	16	Misc.[b]	2	8	3	14
Total	341	253	74.2	195	58	29.7	16	134	28		4	17	4	25

[a] Markers showing polymorphisms between the parental lines, D1s-15s-10s and J1s-14s-23s, including three large (>0.1 kbp) InDel markers, ACAAJ79, ACAAX07 and TC1952

[b] Marker names are headed with "TC" or EST clone names such as ACA and CF

[c] Wako et al. (2002b)

[d] McCallum et al. (2001), Gökçe et al. (2002), Kuhl et al. (2004) and Martin et al. (2005)

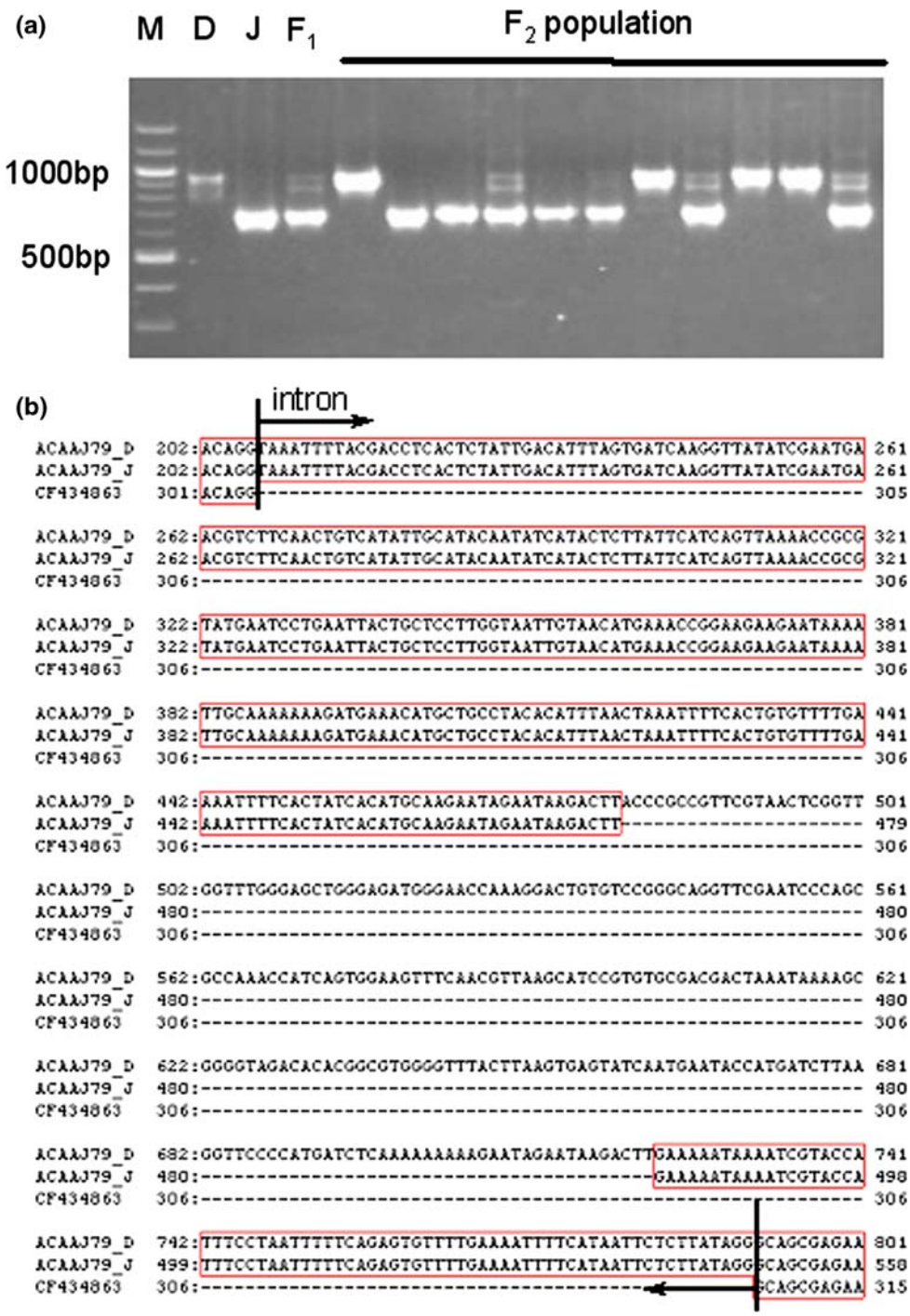

Fig. 1 DNA polymorphisms of bulb onion EST-derived markers within F_2 individuals of bunching onion. *M* 100 bp ladder; *D* D1s-15s-10s; *J* J1s-14s-23s. **a** A large InDel marker ACAAJ79. **b** DNA sequence alignment of a part of the ACAAJ79 locus; PCR products from the bunching onion parental lines, D1s-15s-10s (ACAAJ79_D) and J1s-14s-23s (ACAAJ79_J), and the bulb onion EST sequence (CF434863) which was submitted to GenBank for bulb onion cDNA clone of ACAAJ79. A large deletion (243 bp) was detected in a putative intron region in the parental line 'J'

For a small InDel marker, TC0678 (containing 8 bp InDel), primer sets were redesigned to differentiate the parental alleles clearly (data not shown). Of the 54-containing loci, 17 were directly converted to CAPS markers, and the other 4 loci (ACAEK46, ACAER72, CF439394 and TC2026) were successfully converted to dCAPS markers (data not shown).

We thus obtained 25 polymorphic InDel, CAPS or dCAPS markers (Table 2). These markers, except for ACAAX07, AOB272 and TC1891, segregated codominantly in the F_2 population.

Linkage map of bunching onion

The genetic map we constructed for the F_2 population consists of 17 linkage groups with 229 SSRs (212 bunching onion SSRs and 17 bulb onion SSRs) and 25 bulb onion-derived non-SSR markers (4 InDels, 17 CAPSs and 4

dCAPSs) covering 2,069 cM (Fig. 2). Of the markers located on this map, 151 bunching onion SSRs, 11 bulb onion SSRs and 5 bulb onion-derived non-SSRs were newly developed in this study (Tables 1, 2; Suppl. Tables 1, 2, 3). The other polymorphic markers were not mapped mainly due to distorted segregation or absence of significant linkages with other markers. The average marker distance was 8.1 cM. Ten linkage groups in our map corresponded to the 9 groups of a previously reported linkage map of bunching onion (Ohara et al. 2005) with 11 SSR and 2 CAPS markers in common (Fig. 2).

Assignment of bunching onion linkage groups to their corresponding *A. cepa* chromosomes

Thirty of the 212 bunching onion SSR markers located on our bunching onion map were examined for their chromosomal location using Shigyo et al.'s complete set of bunching onion–shallot monosomic addition lines. Of them, only 8 (AFAA02D08, AFB20G05, AFS015, AFS039, AFS104, AFS145, AFS149 and AFRA11F11) could be assigned to corresponding *A. cepa* chromosomes (Table 3). The others could not be assigned due to their poor amplifiability in shallot, monomorphism between bunching onion and shallot, or inconsistent polymorphisms (probably caused by some residual heterozygousity in the shallot and/or bunching onion lines used for developing the monosomic addition lines).

A total of 42 bulb onion-derived markers (17 SSRs, 4 InDels, 17 CAPSs and 4 dCAPSs) were mapped on 13 of the 17 linkage groups in our bunching onion map (Fig. 2). Of them, 13 markers were already mapped on a *A. cepa* chromosome map by Martin et al. (2005). We independently identified relevant *A. cepa* chromosomes for 3 of these markers, that is, ACC013, ACC033 (named as API40 and AJB64, respectively, in Martin et al.'s bulb onion map) (Wako et al. 2002b) and ACADR60 (in this study).

Of the 29 other bulb onion markers, we previously identified relevant *A. cepa* chromosomes for 4 markers (ACC008, ACC015, ACC043 and AMS14) (Wako et al. 2002b, Masuzaki et al. 2006). In the present study, we identified relevant *A. cepa* chromosomes for 3 other markers (ACE039, ACE044 and TC1010; Table 3). Besides these newly assigned markers, ACADR60, which Martin et al. (2005) assigned to the 6C chromosome, was assigned to 2C on the basis of our data (Fig. 3). Five markers could not be assigned to relevant *A. cepa* chromosomes due to monomorphism between bunching onion and shallot. The remaining 17 bulb onion-derived markers on our bunching onion map have not, so far, been examined for relevant chromosomes.

Twelve linkage groups in our bunching onion map were connected to 7 *A. cepa* chromosomes (Chrs. 1C–6C and 8C) with 8 bunching onion-derived SSR markers and 20 bulb onion-derived markers (1 genomic SSR, 8 EST-SSRs, 1 InDel, 9 CAPSs and 1 dCAPS) (Table 3).

Assignment of bunching onion linkage groups to their relevant chromosomes

Of the 212 bunching onion SSR markers on our map, 139 were examined for their chromosome assignment using the allotriploid-bunching onion single-alien deletion lines. Seventy markers were not amplified in shallot, and 39 of them were assigned to *A. fistulosum* chromosomes on the basis of the absence of bunching onion-specific bands in one of the deletion lines. The other 69 markers were amplifiable in shallot. Of these, 55 were polymorphic between bunching onion and shallot, and 38 of them were assigned to *A. fistulosum* chromosomes (Fig. 4a, b). In addition, the relevant bunching onion chromosomes for 2 bulb onion EST-derived SSR markers (ACM096 and ACE113) were determined with the deletion lines.

Eleven bunching onion linkage groups were assigned to 5 chromosomes (Chrs. 1F, 4F, 6F, 7F and 8F) with 77 bunching onion- and 2 bulb onion-derived SSR markers (Table 4).

Discussion

In our previous study, we constructed a low-density bunching onion linkage map covering 947 cM mainly based on amplified fragment length polymorphism markers (Ohara et al. 2005). In the present study, we constructed a new bunching onion linkage map covering 2,069 cM (Fig. 2). This is the first report of linkage map mainly based on SSR markers in the genus *Allium*. Compared with previously reported bulb onion maps (694 cM, van Heusden et al. 2000; 1,907 cM, Martin et al. 2005), our map is thought to be a standard map in bunching onion. This linkage map will be useful for genetic studies such as QTL analysis of agronomic traits in bunching onion.

Here, we also demonstrated that many bulb onion-derived markers were useful for mapping in bunching onion (Tables 1, 2). Among bulb onion EST-derived markers, the frequency of informative (i.e., polymorphic between the parental lines of bunching onion) markers were higher in those with InDels and/or SNPs (29.2%) than in those with SSRs (11.0%) (Tables 1, 2). We developed or identified 75 polymorphic markers (18 SSRs and 57 InDels and/or SNPs) from 558 bulb onion EST-derived primer sets, and 42 of them were located on the bunching onion map (Fig. 2).

From 11,008 bulb onion EST sequences (Kuhl et al. 2004), we found 1,090 sequences highly homologous to

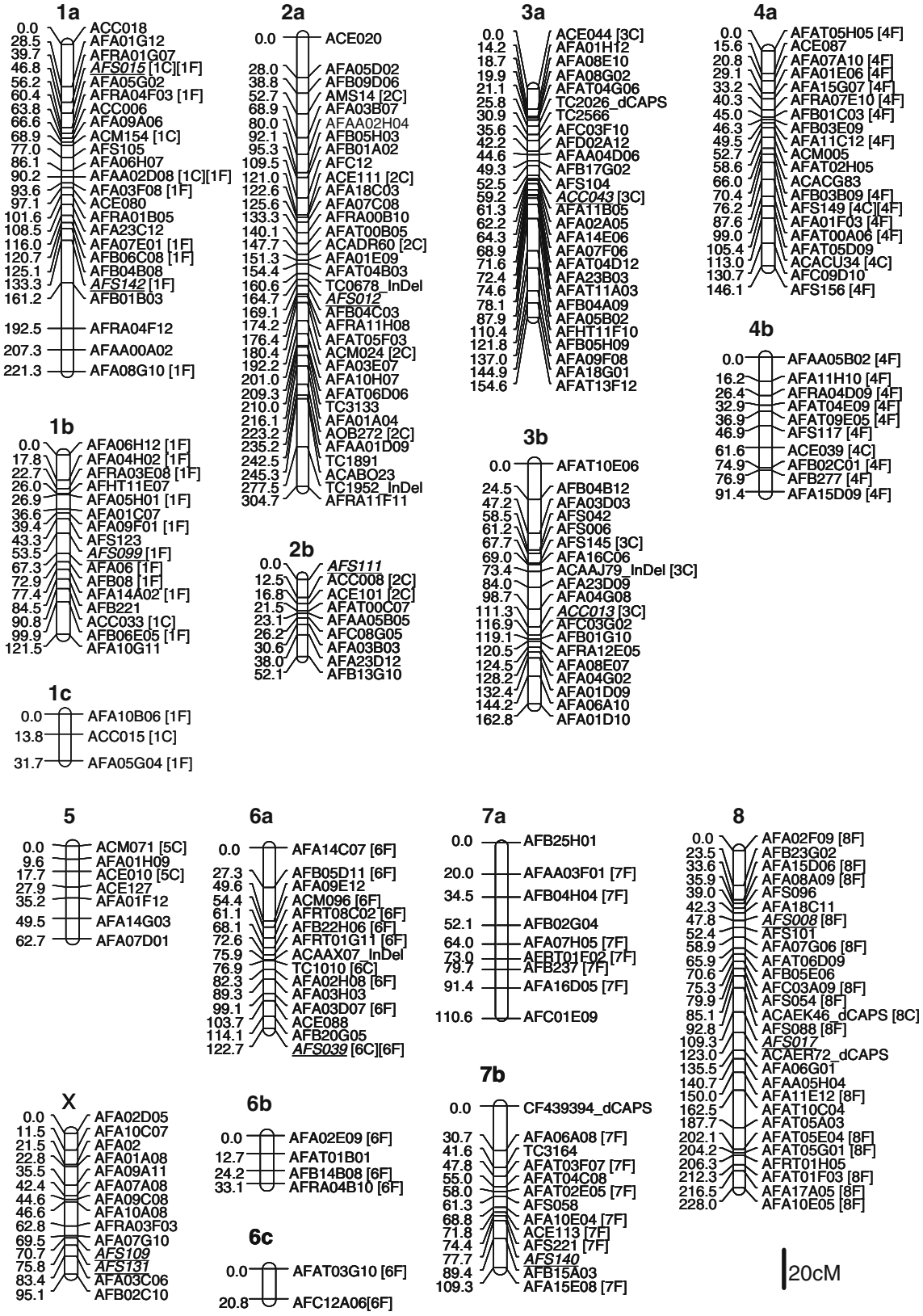

Fig. 2 Bunching onion chromosome map consisting of 17 linkage groups with 229 SSRs (212 bunching onion SSRs and 17 bulb onion SSRs) and 26 bulb onion-derived non-SSR markers (4 InDels, 17 CAPSs and 4 dCAPSs) covering 2,069 cM. The genetic markers used to assign linkage groups to their relevant chromosomes are marked with [1C] to [8C] (assigned to *A. cepa* chromosomes) and [1F] to [8F] (to *A. fistulosum* chromosomes). Markers located on a previously reported bunching onion linkage map (Ohara et al. 2005) are indicated in *italic* with *underlines*

Table 3 List of markers with the chromosome identity determined in *A. cepa*

Marker	Origin	Marker type	Linkage group in *A. fistulosum*	Chromosome number in *A. cepa*		Corresponding marker in *A. cepa* map	Accession No.
				Addition lines[a]	*A. cepa* map[b]		
ACM154	Bulb onion EST	SSR	1a		1	ACM154	CF451414
AFAA02D08	Bunching onion genomic	SSR	1a	1[e]		–	–
AFS015	Bunching onion genomic	SSR	1a	1[e]		–	–
ACC033	Bulb onion EST	CAPS	1b	1[d]	1	AJB64	AA451549
ACC015	Bulb onion EST	CAPS	1c	1[d]		–	ALCLECTINA
ACADR60	Bulb onion EST	CAPS	2a	2[e]	6	ACADR60	CF437227
ACE111	Bulb onion EST	SSR	2a		6	ACM177	CF445960
ACM024	Bulb onion EST	SSR	2a		6	ACM024	CF446873
AFRA11F11	Bunching onion genomic	SSR	2a	2[e]		–	–
AMS14	Bulb onion genomic	SSR	2a	2[c]		–	–
AOB272	Bulb onion EST	CAPS	2a		2	AOB272-E1- 10.0/12.0	AA451592
ACC008	Bulb onion EST	CAPS	2b	2[d]		–	AF218356
ACE101	Bulb onion EST	SSR	2b		6	ACM066	CF437209
ACC043	Bulb onion EST	CAPS	3a	3[d]		–	AJ006067
ACE044	Bulb onion EST	SSR	3a	3[e]		ACM078	CF436576
AFS104	Bunching onion genomic	SSR	3a	3[e]		–	–
ACAAJ79_InDel	Bulb onion EST	InDel	3b		3	ACAAJ79	CF434863
ACC013	Bulb onion EST	CAPS	3b	3[d]	3	API40	AA451549
AFS145	Bunching onion genomic	SSR	3b	3[e]		–	–
ACACU34	Bulb onion EST	CAPS	4a		4	ACACU34	CF449808
AFS149	Bunching onion genomic	SSR	4a	4[e]		–	–
ACE039	Bulb onion EST	SSR	4b	4[e]		–	CF442638
ACE010	Bulb onion EST	SSR	5		5	ACM133	CF436768
ACM071	Bulb onion EST	SSR	5		5	ACM071	CF449595
TC1010	Bulb onion EST	CAPS	6a	6[e]		–	CF440857/CF449499
AFB20G05	Bunching onion genomic	SSR	6a	6[e]		–	–
AFS039	Bunching onion genomic	SSR	6a	6[e]		–	–
ACAEK46_dCAPS	Bulb onion EST	dCAPS	8		8	ACAEK46	CF451695

[a] Complete set of alien addition lines of *A. fistulosum* carrying single chromosomes of shallot (*A. cepa*) (Shigyo et al. 1996)

[b] Reported by Martin et al. (2005)

[c] Reported by Masuzaki et al. (2006)

[d] Reported by Wako et al. (2002b)

[e] Identified in the present study

rice unigene cDNA sequences, and designed 93 primer sets for intron spanning EST-derived markers for bunching onion. We did not design primer sets for the other sequences mainly due to the large sizes (>1 kb) of introns in corresponding rice gene sequences and substantial nucleotide differences at intron-flanking sequences between bulb onion and rice. Kuhl et al. (2004) reported a lower average similarity at intron regions (39%) between bulb onion and rice than at cording (78%). We found no correlation ($r = 0.07$) between the PCR amplicon sizes of intron-span-

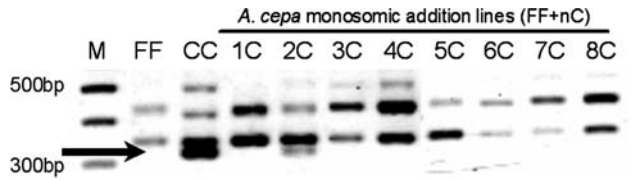

Fig. 3 Direct identification of relevant *A. cepa* chromosomes for the bulb onion EST-derived CAPS marker ACADR60 digested by *Taq*I by using Shigyo et al's complete set of bunching onion–shallot monosomic addition lines. *Arrows* show shallot-specific DNA fragments. The marker is located on 2C chromosome

ning markers in bunching onion and the known sizes of corresponding rice genomic regions (data not shown). In addition, Kuhl et al. (2004) reported that the average GC content at the third codon position was very different between bulb onion (40.9%) and rice (61.8%). In this regard, it will be possible to design many additional primer sets for EST-markers for bunching onion by neglecting synonymous third codon differences between bulb onion and rice. Therefore, many more bulb onion EST-derived markers may subsequently be added to the bunching onion map.

Forty-two bulb onion-derived markers, including 1 genomic SSR, were located on 13 of the 17 linkage groups in our bunching onion map (Fig. 2). In the bulb onion map reported by Martin et al. (2005), AOB272 was located on Chr. 2C and 4 other markers (ACADR60, ACM024, ACM066 and ACM177) were located on Chr. 6C. Masuzaki et al. (2006) directly identified the relevant *A. cepa* chromosome (2C) for AMS14. In our bunching onion map, these markers were located on either of the linkage groups of LG2a [ACADR60, ACM024, ACM177 (ACE111 in our map) and AMS14] or LG2b [ACM066 (ACE101 in our map)]. Of them, 3 markers (ACE101, ACE111 and ACM024) were monomorphic between bunching onion and shallot (data not shown). In the present study, we directly identified the relevant *A. cepa* chromosome for ACADR60 and ACC008, which was tightly linked to ACE101, using Shigyo et al.'s shallot monosomic addition lines (Fig. 3). In addition, ACM024 located on LG2a and ACE111 on LG2b in our bunching onion map were linked to AOB272 in another bulb onion population (J. McCallum, personal communication). Therefore, we conclude that LGs 2a and 2b in our bunching onion map correspond to *A. cepa* chromosome 2 (Table 2).

Bunching onion and bulb onion belong to the section *Cepa* and have the same chromosome number ($2n = 16$), although they were classified into different alliances—*Altaicum* alliance and *Cepa* alliance (Fritsch and Friesen 2002), respectively. By using the monosomic additions and/or sin-

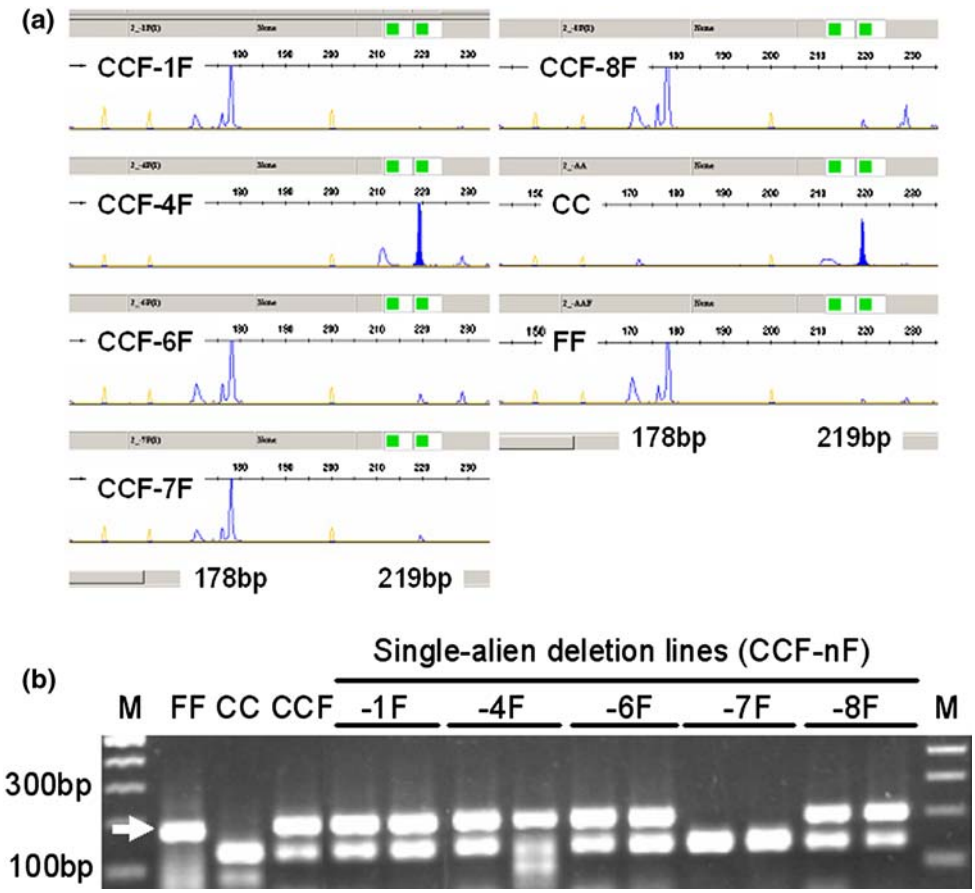

Fig. 4 Direct identification of relevant *A. fistulosum* chromosomes for bunching onion SSR markers AFRA07E10 (**a**) and AFA06A08 (**b**) by using allotriploid-bunching onion single-alien deletion lines. These markers are located on 4F (AFRA07E10) and 7F (AFA06A08) chromosomes, respectively

Table 4 List of SSR markers with the chromosome identity determined in *A. fistulosum*

Chromosome No.[a]	Linkage group	SSR markers				
1F	1a	AFA03F08	AFA07E01	AFA08G10	AFAA02D08	AFB06C08
		AFS015	AFS142	AFRA04F03		
	1b	AFA04H02	AFA05H01	AFA06	AFA06H12	AFA09F01
		AFA14A02	AFB06E05	AFB08	AFRA03E08	AFS099
	1c	AFA05G04	AFA10B06			
4F	4a	AFA01E06	AFA01F03	AFA11C12	AFA15G07	AFAT00A06
		AFAT05H05	AFB01C03	AFB03B09	AFB03E09	AFRA07E10
		AFS149	AFS156			
	4b	AFA11H10	AFA15D09	AFAA05B02	AFAT04E09	AFAT09E05
		AFB02C01	AFB277	AFRA04D09	AFS117	
6F	6a	ACM096[b]	AFA02H08	AFA14C07	AFB05D11	AFB22H06
		AFRT01G11	AFRT08C02	AFS039		
	6b	AFA02E09	AFB14B08	AFRA04B10		
	6c	AFAT03G10	AFC12A06			
7F	7a	AFA07H05	AFA16D05	AFAA03F01	AFB04H04	AFB237
	7b	ACE113[b]	AFA06A08	AFA10E04	AFA15E08	AFAT02E05
		AFAT03F07	AFS221			
8F	8	AFA07G06	AFA08A09	AFA10E05	AFA11E12	AFA15D06
		AFA17A05	AFAT01F03	AFAT05E04	AFAT05G01	AFC03A09
		AFS008	AFS054	AFS088		

[a] Determined by using allotriploid-bunching onion single-alien deletion lines (Hang et al. 2004; Yaguchi et al. 2008)

[b] Bulb onion EST-derived SSR marker

gle-alien deletions, the direct comparisons of the relevant chromosomes for 103 markers consistently assigned 16 of the 17 linkage groups in our *A. fistulosum* map to the 8 basic chromosomes of *A. cepa*; 28 markers were assigned to *A. cepa* chromosomes, and 79 to *A. fistulosum* chromosomes, with 4 bunching onion SSR markers (AFAA02D08, AFS015, AFS039 and AFS149) assigned to both *A. cepa* and *A. fistulosum* chromosomes (Fig. 2; Tables 3, 4). This newly developed set of anchor markers comprises 81 bunching onion SSRs, 11 bulb onion SSRs and 11 bulb onion non-SSRs (1 InDel, 9 CAPSs and 1 dCAPS). We therefore conclude that a very high level of macrosynteny exists between these two species.

The remaining linkage group, temporarily named as LG X, could not be assigned to any chromosome. We consider two possible explanations for this: (1) the incompleteness of the set of allotriploid-bunching onion single-alien deletion lines suggests that LG X might belong to any of the 2F, 3F or 5F chromosome; (2) all the markers located on LG X were bunching onion-derived SSR markers, which might have resulted in the exclusive amplification of bunching onion genome regions. In another bunching onion F_2 population, some markers located on bunching onion LG X were loosely linked to ACE010 (ACM133 in Martin et al. 2005, assigned to *A. cepa* Chr. 5) (unpublished data). The relevant chromosome of LG X would be identified by mapping new bulb onion-derived markers and/or by integrating different bunching onion linkage maps with common markers.

As compared with bulb onion, bunching onion is suitable for genetic studies because this crop is annual and has a 28% smaller genome size (Ricroch et al. 2005). Genome synteny is, as mentioned above, highly conserved between bunching onion and bulb onion. However, it was reported that a minimum of two heteromorphic bivalents were observed in meiotic analysis of interspecific hybrids between bunching onion and bulb onion (Peffley 1986), suggesting that chromosomal mutations such as translocation and inversion might be involved in the speciation between *A. fistulosum* and *A. cepa*. For detailed studies of such chromosomal mutations, microsynteny should be evaluated at the genome-wide level by plotting many more common markers onto the maps of both species.

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