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ORIGINAL PAPER

Direct determination of the chromosomal location of bunching onion and bulb onion markers using bunching onion–shallot monosomic additions and allotriploid-bunching onion single alien deletions

Hikaru Tsukazaki · Ken-ichiro Yamashita · Shigenori Yaguchi · Koichiro Yamashita · Takuya Hagihara · Masayoshi Shigyo · Akio Kojima · Tadayuki Wako

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Abstract To determine the chromosomal location of bunching onion (*Allium fistulosum* L.) simple sequence repeats (SSRs) and bulb onion (*A. cepa* L.) expressed sequence tags (ESTs), we used a complete set of bunching onion–shallot monosomic addition lines and allotriploid bunching onion single alien deletion lines as testers. Of a total of 2,159 markers (1,198 bunching onion SSRs, 324 bulb onion EST–SSRs and 637 bulb onion EST-derived non-SSRs), chromosomal locations were identified for 406 markers in *A. fistulosum* and/or *A. cepa*. Most of the bunching onion SSRs with identified chromosomal locations showed polymorphism in bunching onion (89.5%) as well as bulb onion lines (66.1%). Using these markers, we constructed a bunching onion linkage map (1,261 cM), which consisted of 16 linkage groups with 228 markers, 106 of which were newly located. All linkage groups of this map were assigned to the eight basal *Allium* chromosomes. In this study, we assigned 513 markers to the eight chromosomes of *A. fistulosum* and *A. cepa*. Together with 254 markers previously located on a separate bunching onion map, we have identified chromosomal locations for 766 markers in total. These chromosome-specific markers will be useful for the intensive mapping of desirable genes or QTLs for agricultural traits, and to obtain DNA markers linked to these.

Keywords *Allium cepa* · *Allium fistulosum* · Chromosome identity · Linkage map · Monosomic addition lines · Single alien deletion lines

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H. Tsukazaki (✉) · K. Yamashita · A. Kojima · T. Wako
National Institute of Vegetable and Tea Science (NIVTS), NARO, 360 Ano-Kusawa, Tsu, Mie 514-2392, Japan
e-mail: tsuka@affrc.go.jp

S. Yaguchi · M. Shigyo
Department of Biological and Environmental Science, Faculty of Agriculture, Yamaguchi University, 1677-1 Yoshida, Yamaguchi, Yamaguchi 753-8515, Japan

Present Address:
S. Yaguchi
Toricon Co., Ltd, Nakano 3825-8, Ohnan, Ohchi, Shimane 696-0102, Japan

K. Yamashita · T. Hagihara
Hokuren Agricultural Research Institute, Minami2, Higashi9, Naganuma, Hokkaido 069-1316, Japan

Abbreviations

EST	Expressed sequence tag
InDel	Insertion–deletion
SNP	Single nucleotide polymorphism
SSR	Simple sequence repeat

Introduction

In the genus *Allium*, bulb onion (*A. cepa* L.), garlic (*A. sativum* L.) and bunching onion (*A. fistulosum* L.) are the most economically important species in the world. Of them, bulb onion and bunching onion belong to the section *Cepa* in the genus *Allium*. Although these species have the same number of chromosomes ($2n = 2x = 16$), the genome composition was different (CC for bulb onion and FF for bunching onion). In Japan, bunching onion is more

important than other *Allium* crops, as it has the highest annual output among *Allium* species (MAFF 2008). However, despite the economic importance of bunching onion and bulb onion, the genetics of these species are poorly understood and molecular-based approaches need to be utilized to clarify such agronomic characteristics. By determining these genetic characteristics, it will be possible to utilize comparative genomics approaches between bunching onion and bulb onion, and to conduct molecular breeding programs in both crops.

In bulb onion, hundreds of expressed sequence tag (EST)-derived simple sequence repeat (SSR), EST-derived single nucleotide polymorphism (SNP) and insertion–deletion (InDel) markers have been developed from large-scale sequencing of ESTs (Kuhl et al. 2004; Martin et al. 2005). In bunching onion, we isolated more than 2,000 SSR clones from a genomic library and 10 SSR-enriched DNA libraries (Song et al. 2004; Tsukazaki et al. 2007). Using these markers, linkage maps have been developed for bulb onion (King et al. 1998; Martin et al. 2005) and bunching onion (Ohara et al. 2005; Tsukazaki et al. 2008). In this way, thousands of markers have been developed from bunching onion and bulb onion. Nevertheless, these markers have not been sufficiently utilized for genetic studies due to the limited number of them that are mapped.

Bunching onion and bulb onion belong to the section *Cepa* in the genus *Allium* and have the same number of chromosomes, although they are classified into different alliances: *Altaicum* alliance and *Cepa* alliance (Fritsch and Friesen 2002), respectively. Although hybrids between these species have been obtained (Emsweller and Jones 1935a, b; Maeda 1937), these hybrids showed severe sterility. To overcome this problem, progenies from interspecific hybrids were successfully produced by using amphidiploids or allotriploids (Levan 1941; Tashiro 1984).

Alien chromosome additions, such as monosomic or disomic additions, and deletions (e.g., nulli-tetrasomics) are useful tools in plant genetic research and breeding. In several crops, these lines have been used for chromosomal assignment of molecular markers or linkage groups (Chatelat et al. 1998; Fox et al. 2001; Kam-Morgan et al. 1989; Song et al. 2005) and morphological characterization (Mesbah et al. 1997; Budahn et al. 2008).

Peffley et al. (1985) first reported the development of several alien monosomic addition lines ($2n = 16 + 1 = 17$, CC + *n*F) between *A. cepa* and *A. fistulosum* using an interspecific triploid cultivar. Although this was an incomplete set, they identified three isozyme markers, *Adh-1*, *Idh-1* and *Pgm-1*, located on the different *A. fistulosum* chromosomes. Meanwhile, Shigyo et al. (1996) developed a complete set of bunching onion–shallot (*A. cepa* L. Aggregatum group) monosomic addition lines ($2n = 17$, FF + 1C to 8C; note that Shigyo et al. (1996) used the symbol "A" instead of "C" for shallot chromosomes). Each monosomic addition line displays distinct morphological and biochemical characteristics (Shigyo et al. 1997). These complete addition lines have also been effectively used for determining the chromosomal locations of genes involved in flavonoid biosynthesis (Masuzaki et al. 2006a), sulfur assimilation (McCallum et al. 2007) and sucrose metabolism (Yaguchi et al. 2008). In addition, Hang et al. (2004a) and Yaguchi et al. (2009) developed hypo-allotriploids, in which one of the alien chromosomes was 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; denoted as 'single alien deletions'), and evaluated their morphological and biochemical characteristics (Hang et al. 2004b; Yaguchi et al. 2009). By using these monosomic additions and single alien deletions, linkage maps have also been simultaneously anchored to bulb onion and bunching onion chromosomes (van Heusden et al. 2000b; Martin et al. 2005; Tsukazaki et al. 2008).

The objective of the present study was to directly assign a large number of bunching onion and bulb onion molecular markers to the chromosomes of *A. fistulosum* and *A. cepa* using these alien chromosome addition and deletion lines. To evaluate the degree of polymorphisms, bunching onion SSRs assigned to each of the *Allium* chromosomes were applied to the both bunching onion and bulb onion lines. In addition, we constructed a new bunching onion map and compared it to a previously developed bunching onion map.

Materials and methods

SSR markers

The bunching onion (*A. fistulosum* L.) cultivar 'Kujo-hoso' and the shallot (*A cepa* L.) cultivar 'Chiang mai' were used for detecting polymorphisms between the two species. Total DNA was extracted from each plant according to the method described by Song et al. (2004).

We screened 1,198 primer sets previously developed from bunching onion genomic SSR clones (Song et al. 2004; Tsukazaki et al. 2007, 2008). The chromosomal identities of these SSR markers have not yet been determined. PCR conditions were the same as those used for the cleaved amplified polymorphic sequence (CAPS) analysis by Ohara et al. (2005). PCR products were separated by 3% (w/v) agarose gel electrophoresis and screened for polymorphisms between bunching onion and shallot.

For the bulb onion EST-derived SSR markers, we screened 115 markers reported by Kuhl et al. (2004), Martin et al. (2005), Jakše et al. (2005) and McCallum

et al. (2008). We also obtained 209 primer sets based on bulb onion EST sequences from the Dana-Farber Cancer Institute (DFCI) *A. cepa* Gene Index v.2.0 (http://compbio.dfci.harvard.edu/tgi/cgi-bin/tgi/gimain.pl?gudb=onion) according to Tsukazaki et al. (2008). Since the degree of polymorphisms in bulb onion EST–SSRs is less than that in bunching onion genomic SSRs (Tsukazaki et al. 2007, 2008), PCR fragments were labeled with fluorescent dideoxynucleotides according to the method of Kukita and Hayashi (2002), loaded on a capillary DNA sequencer (ABI3730; Applied Biosystems, CA, USA), and polymorphisms were detected using GeneMapper ver. 3.0 software (Applied Biosystems).

Non-SSR markers

We screened 246 primer sets previously developed from bulb onion ESTs (McCallum et al. 2001; Kuhl et al. 2004; Martin et al. 2005; Jakše et al. 2005; McCallum et al. 2008). We developed 361 intron-spanning EST-derived markers from the DFCI bulb onion EST database according to Tsukazaki et al. (2008). In addition, we also screened 30 *Allium–Oryza* conserved intron-spanning primers (CISPs) developed by Lohithaswa et al. (2007).

PCR fragments from bunching onion 'Kujo-hoso' and shallot 'Chiang mai' were separated on 2% (w/v) agarose gels. When distinct allele differences (>0.1 kbp in fragment size) were identified, the primers were applied to both monosomic additions and single alien deletions. For identification of small InDels, each PCR product (<0.4 kbp in fragment size) was selected, labeled and loaded on a capillary DNA sequencer as described above.

For identification of single nucleotide polymorphisms (SNPs) for loci that were not polymorphic by gel electrophoresis, each PCR product (>0.4 kbp in fragment size) from bunching onion and shallot at 217 loci, was purified using the MultiScreen PCR 96 system (Millipore, MA, USA), and sequenced with the DNA sequencer. 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).

Chromosomal assignment of markers

To determine the chromosomal identities of 619 markers (255 bunching onion SSRs and 364 bulb onion ESTs), we used a complete set of monosomic addition lines ($2n = 16 + 1$, 17; FF + 1C to FF + 8C) developed by Shigyo et al. (1996). We also applied 912 markers (690 bunching onion SSRs and 222 bulb onion EST markers) to a set of single alien deletions ($2n = 24 - 1$, 23; CCF-1F, CCF-4F, CCF-6F, CCF-7F and CCF-8F) developed by Hang et al. (2004a) and Yaguchi et al. (2009).

When the primers amplified fragments only in shallot, the markers were applied to a set of monosomic additions to determine chromosome identities by amplifying the shallot-specific band. In contrast, when the primers amplified only in bunching onion, the markers were applied to a set of single alien deletions to determine chromosome identities by the absence of a bunching onion-specific band. In cases where there were fragments of different size between bunching onion and shallot, the markers were applied to both sets of monosomic additions or single alien deletions.

Polymorphism within bunching onion and bulb onion lines

Bunching onion SSR markers with determined chromosome identities were used to investigate polymorphisms within both bunching onion and bulb onion lines. For the bunching onion, DNA polymorphisms at 153 SSR loci (Suppl. Table 1) were evaluated in 'Kujo-hoso' and 10 bunching onion inbred lines: C1s-11s-25s-7 (C), D1s-15s-10s-8 (D), F1s-7s-17s-3s-1 (F), J1s-14s-23s-9 (J), K1s-5s-2s-2s-4s-5 (K), Cho1s-1s-2s-2s-8s (Cho), Sa48-10s-7ic-3s-2ic-7s-1s-2s (Sa02), Sa48-10s-7ic-3s-2ic-3s-1s-9s (Sa03), T23-5s-1s-2s-5 (T02) and T26-4s-2s-2s (T03). The inbred lines C, D, Cho, Sa02 and Sa03 belong to the "Senju" group, F to "Kaga" and J to "Kujo". Two inbred lines, T02 and T03, were derived from a cross between "Shimonita/Kujo hybrid" and "Senju" lines, and the inbred line K is derived from a Taiwanese ever-flowering cultivar "Kitanegi". For the bulb onion, DNA polymorphisms at 56 SSR loci (Suppl. Table 1) were evaluated in shallot 'Chiang mai' and six bulb onion inbred lines: BP4-1, BP2-4, FP1-5, FP5-3, SP1-4 and SP5-6. All bulb onion inbreds except for BP2-4 belong to the long-day and pungent type. BP2-4 belongs to the intermediate type. Fifty-three loci were also investigated for polymorphisms between bunching onion lines. PCR fragments were fluorescent labeled, loaded and analyzed as described above.

To assess the degree of DNA polymorphism at each SSR locus in bunching onion and bulb onion, the number of alleles and the polymorphism information content (PIC) were evaluated. The PIC value was calculated for each SSR locus according to the following formula (Anderson et al. 1993): $\mathrm{PIC} = 1 - \sum_{i=1}^{k} P_i^2$, where k is the total number of alleles detected at a locus and P_i is the frequency of the ith allele in the either set of 11 bunching onion lines and seven bulb onion lines investigated.

Construction of bunching onion linkage map

An SSR marker, AFAT06C11, assigned to chromosome 5C, was added to a previous bunching onion map (DJ map; Tsukazaki et al. 2008).

A separate mapping population, consisting of a total of 119 individuals of the F_2 progeny derived from a cross between two bunching onion inbred lines, T03 and Sa03, was used. The F_2 mapping population (denoted SaT03) was the selfed progeny of a single F_1 plant between these parents. 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 4.0 was used to group and order the markers. The numbers of linkage groups were determined by comparison with the DJ map.

Results

SSR markers

Of 1,198 bunching onion genomic SSR primer sets, 756 (63.1%) detected polymorphisms between bunching onion and shallot (Table 1). Most of the polymorphic markers (501/756 markers, 66.3%) could not be amplified well in shallot. The remaining markers amplified fragments of different size between bunching onion and shallot. Markers amplified only in shallot were not obtained.

Of the bulb onion EST-derived SSRs, 235 of 324 primer sets (72.5%) showed polymorphisms between bunching onion and shallot (Table 1). Of the polymorphic markers, 57.4% (135 markers) amplified different size PCR products.

We thus obtained 991 SSR markers showing polymorphic patterns between bunching onion and shallot, and applied 742 bunching onion SSRs and 99 bulb onion EST-derived SSR markers to the sets of monosomic additions and/or single alien deletions (Table 1).

Non-SSR markers

Of 637 bulb onion EST-derived non-SSR primer sets, 206 detected polymorphisms between bunching onion and shallot (Table 1). Attempts were made to assign 202 of these markers to chromosomes. The frequency of polymorphic markers (32.3%) was less than that in both bunching onion (63.1%) and bulb onion EST-derived SSRs (72.5%). Next, we attempted to detect SNPs between PCR fragments of bunching onion and bulb onion. From a sequence comparison of 217 loci, 162 (74.7%) were polymorphic between bunching onion and shallot: InDels (1–20 bp) at 18 loci, SNPs at 151 loci, and 7 loci

Table 1 Numbers of markers showing polymorphisms between bunching onion and shallot

Marker	No. of primer sets tested	Polymorphic between bunching onion and shallot[a]						Amplified only in shallot	(%) in primer set tested	(%) in polymorphic primer set	Total	(%) in primer set tested	Applied to determine chromosome identities
		Different size of amplicon	(%) in primer set tested	(%) in polymorphic primer set	Amplified only in bunching onion	(%) in primer set tested	(%) in polymorphic primer set						
Bunching onion													
Genomic SSR	1,198	255	(21.3)	(33.7)	501	(41.8)	(66.3)	0	(0.0)	(0.0)	756	(63.1)	742
Bulb onion													
EST-derived SSR	324	135	(41.7)	(57.4)	5	(1.5)	(2.1)	95	(29.3)	(40.4)	235	(72.5)	99
EST-derived non-SSR	637	109	(17.1)	(52.9)	42	(6.6)	(20.4)	55	(8.6)	(26.7)	206	(32.3)	202
Total	2,159	499	(23.1)	(41.7)	548	(25.4)	(45.8)	150	(6.9)	(12.5)	1,197	(55.4)	1,043

[a] Scored by separated PCR products on agarose gel (bunching onion SSRs and bulb onion EST-derived non SSRs) and those loaded on a capillary DNA sequencer (bulb onion EST-derived SSRs). For the bulb onion EST-derived non-SSR markers, PCR products (>0.4 kbp in fragment size) were loaded on a capillary DNA sequencer

containing both types of polymorphism (Table 2). SNPs detected within restriction enzyme sites at 129 loci were converted into CAPS markers and we attempted to assign these to chromosomes. In total, assignments for 331 bulb onion EST-derived non-SSR markers were sought using either set of monosomic additions or single alien deletions.

Chromosomal assignment

A total of 619 markers were applied to the monosomic addition lines to determine their chromosomal locations. For the bunching onion SSRs, 32 of 255 were assigned to chromosomes 1C to 8C (Table 3). For the bulb onion EST-derived markers, 176 of 364 were determined to be located on one of the shallot chromosomes (Table 3). In total, the number of markers assigned to each of the shallot chromosomes ranged from 15 (8C) to 40 (2C).

In addition, a total of 912 markers were examined for their chromosomal assignment using the single alien deletion lines. Of these, 378 markers were also applied to the monosomic additions. For the bunching onion SSRs, 187 of 691 were assigned to the chromosomes of 1F, 4F, 6F, 7F and 8F (Table 3). For the bulb onion EST-derived markers, 31 of 222 were also assigned to bunching onion chromosomes (Table 3). In total, the number of markers assigned to bunching onion chromosomes ranged from 23 (8F) to 65 (1F). We also determined the chromosomal identities of 20 of these markers on shallot chromosomes.

We thus obtained 406 markers for which the chromosomal locations were determined (Table 3; Suppl. Tables 1–3). With 254 markers previously located on the bunching onion DJ map, 660 markers were assigned to the chromosomes.

DNA polymorphisms within bunching onion and bulb onion lines

For bunching onion, the total number of alleles detected in the 11 lines sampled varied from 1 to 11 among the 153

Table 2 InDels and SNPs between bunching onion and shallot

No. of primer sets sequenced	Polymorphic between bunching onion and shallot								Applied to determine chromosome identities
	InDel	(%)	SNP	(%)	Both type	(%)	Total	(%)	
217	18	(8.3)	151	(69.6)	7	(3.2)	162	(74.7)	129

Table 3 Numbers of markers with the chromosomal location determined in *A. cepa* and *A. fistulosum*

	No. of primer sets examined	*A. cepa* (C)/*A. fistulosum* (F) chromosome number								Total
		1C	2C	3C	4C	5C	6C	7C	8C	
Shallot monosomic additions (FF + *n*C)										
A. fistulosum genomic SSR	255	2[a]	5	10	2[a]	6	3[a]	3[a]	1	32
A. cepa EST	364	29[a]	35	23	23[a]	24	14[a]	14[a]	14[a]	176
	No. of primer sets examined	*A. cepa* (C)/*A. fistulosum* (F) chromosome number								
		1F			4F		6F	7F	8F	Total
Bunching onion single-alian deletions (CCF-*n*F)										
A. fistulosum genomic SSR	690	60[a]	–	–	39[a]	–	34[a]	34[a]	20	187
A. cepa EST	222	5[a]	–	–	9[a]	–	7[a]	7[a]	3[a]	31
Total (A)	1,163[b]	91[a]	40	33	68[a]	31	53[a]	53[a]	37[a]	406
		1a, 1b, 1c	2a, 2b	3a, 3b	4a, 4b	5, X[c]	6a, 6b, 6c	7a, 7b	8	Total
Linkage numbers of *A. fistulosum* DJ map (Tsukazaki et al. 2008)										
No. of markers located on the linkage map (B)		43	43	47	30	21	21	22	27	254
No. of markers (total) (A + B)		134	83	80	98	52	74	75	64	660
No. of markers located on SaT03 map (Fig. 2)		43	34	32	27	31	16	23	22	228
No. of markers newly located on SaT03 map (C)		13	20	18	4	22	6	13	10	106
No. of markers (total) (A + B + C)		147	103	98	102	74	80	88	74	766

[a] Including 20 markers which were determined for their chromosome identity in both *A. cepa* and *A. fistulosum*

[b] 378 of these were examined for both sets of monosomic additions and single-alien deletions

[c] Linkage group X was estimated to be assigned to chromosome 5C (Fig. 1)

Table 4 Polymorphisms of bunching onion SSR markers within bunching onion and bulb onion lines

	No. of markers	No. of alleles[a]			PIC			Polymorphic markers (%)[b]
		Min	Max	Average	Min	Max	Average	
Bunching onion (11 lines)	153	1	11	3.7	0.00	0.90	0.50	89.5
Bulb onion and shallot (7 lines)	56	1	5	2.2	0.00	0.95	0.29	66.1

[a] Analyzed by a capillary DNA sequencer

[b] Percentage of polymorphic markers within bunching onion or bulb onion lines (PIC >0)

SSR loci investigated, and the PIC value from 0.0 to 0.9 (Table 4). The average number of alleles per locus and the PIC value were 3.7 and 0.50, respectively. The most polymorphic locus AFB12H10, which was assigned to chromosome 4F, had 11 alleles among the bunching onion lines. In contrast, 16 loci were monomorphic in the 11 lines investigated.

In bulb onion and shallot, the total number of alleles detected in the 7 lines varied from 1 to 6 among the 56 loci, and the PIC value from 0.00 to 0.95 (Table 4). The average number of alleles per locus and the PIC value were 2.2 and 0.29, respectively. An SSR marker, AFAA07C04, with a PIC value of 0.41, successfully segregated in the bulb onion F_2 population (data not shown). Among the 53 markers applied to both bunching onion and bulb onion lines investigated, only one SSR marker, AFAA01H06, was monomorphic in both species.

Linkage map of bunching onion

An SSR marker, AFAT06C11, for which the chromosomal location was determined to be 5C, was mapped to linkage group (LG) X of the DJ map (Fig. 1). We thus determined the chromosomal location of LGX to be on chromosome 5C (Chr.5) and denoted this region Chr.5b.

In the SaT03 population, a genetic map consisting of 16 linkage groups with 228 markers (199 bunching onion SSRs, 8 bulb onion SSRs and 21 non-SSR markers) covering 1,261 cM was constructed (Fig. 2; Suppl Tables 1–3). All linkage groups in this map corresponded to the 15 groups identified in the DJ map with 60 markers (56 bunching onion SSRs, 1 bulb onion EST-derived SSR and 2 bulb onion EST-derived non-SSRs) in common (Fig. 2). Of the markers located on the SaT03 map, chromosomal locations were identified for 62 markers (59 bunching onion SSRs and 3 bulb onion EST-derived non-SSRs; Table 3). The remaining 106 markers (84 bunching onion SSRs, 8 bulb onion EST-derived SSRs and 14 bulb onion EST-derived non-SSRs) were newly located onto this map. Thus, chromosome identities were determined for a total of 766 markers (Table 3).

Discussion

Of a total of 2,159 markers used in this study, 1,197 (55.4%) showed polymorphisms between bunching onion

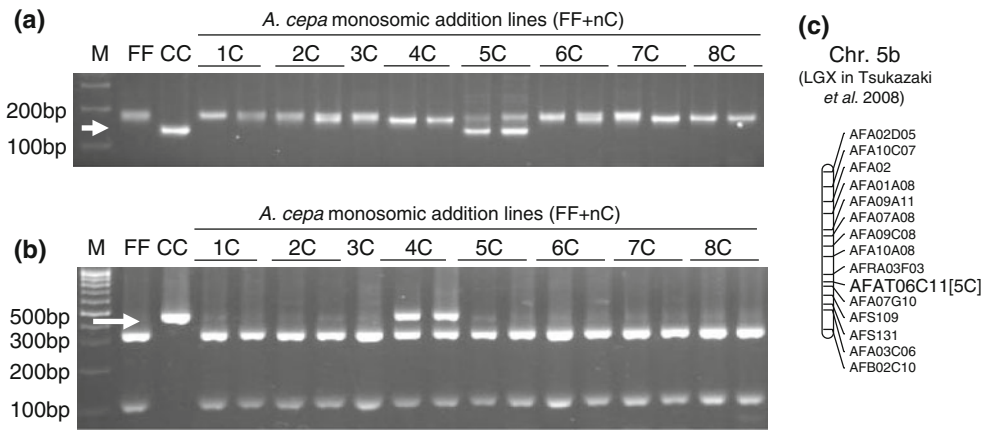

Fig. 1 Direct identification of relevant *A. cepa* chromosomes for the bunching onion SSR marker AFAT06C11 (**a**), bulb onion EST-derived CAPS marker TC3270 digested with *Ssp*I (**b**) and chromosomal location of AFAT06C11 on the DJ map (**c**). *F* bunching onion; *C* shallot; *1C–8C* complete set of bunching onion–shallot monosomic addition lines (FF + 1C to FF + 8C). *Arrows* show shallot-specific DNA fragments. The SSR marker AFAT06C11 is located on chromosome 5C (**a**), and mapped to LGX (**c**). Thus, LGX is assigned to Chr.5 (denoted Chr.5b)

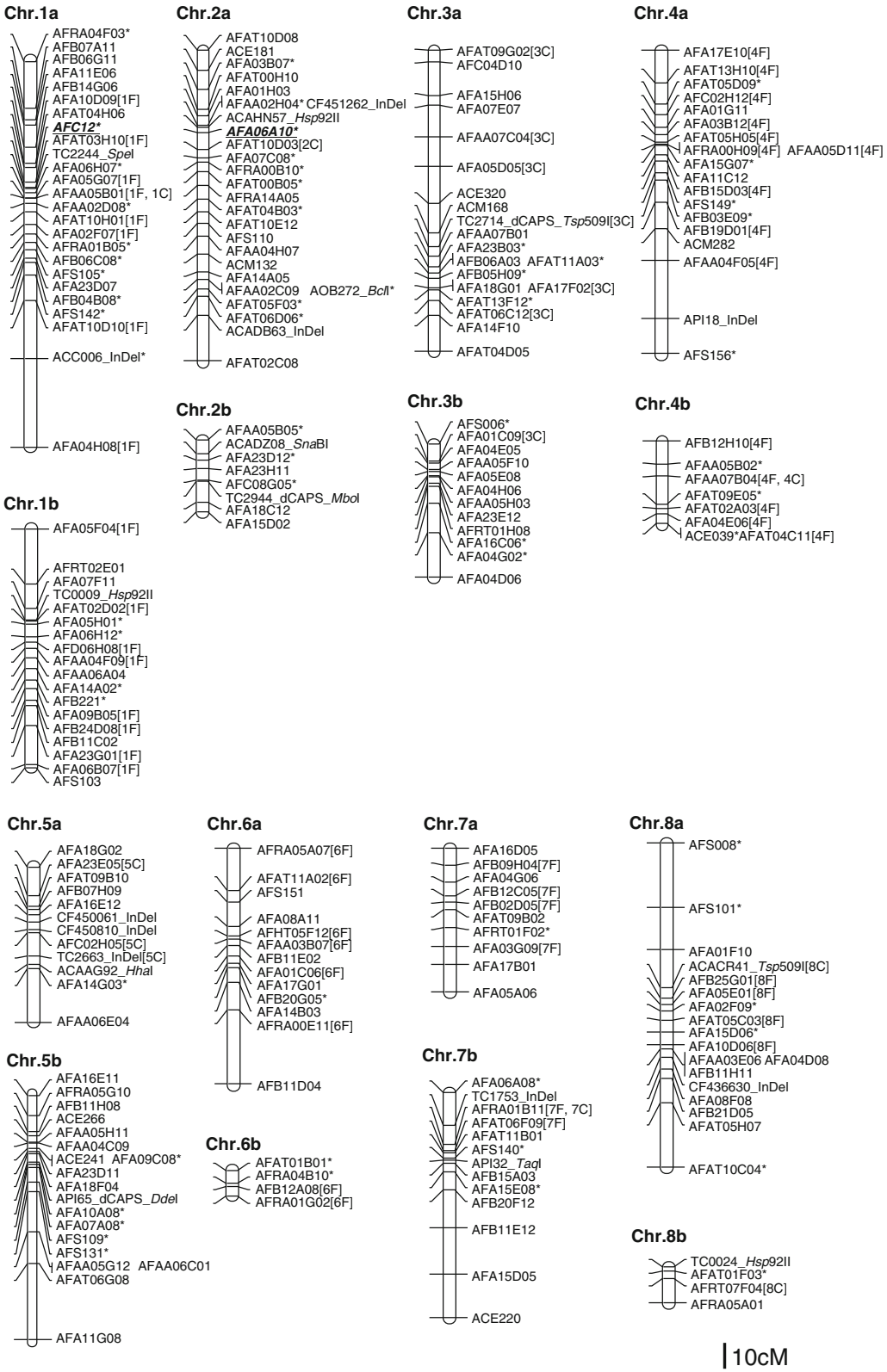

Fig. 2 Bunching onion chromosome linkage map for the SaT03 population, consisting of 16 linkage groups with 232 markers covering 1,261 cM. The genetic markers used to assign linkage groups to their relevant chromosomes are marked with [1C] to [8C] (assigned to *A. cepa* chromosomes) and [1F] to [8F] (*A. fistulosum* chromosomes). Markers located on a previously reported bunching onion linkage map (DJ map; Tsukazaki et al. 2008) are indicated with *asterisks*. Two markers, AFC12 and AFA06A10, whose chromosome locations were discrepant between the two linkage maps, are indicated in *italics* with *underlines*

and shallot. The number of markers that amplified only in bunching onion was the highest (548 markers, 45.8%), followed by markers that amplified different fragment sizes in bunching onion and shallot (499 markers, 41.7%). The frequency of markers that amplified only in shallot was low (120 markers, 12.5%). This was mainly due to the difference of marker sources (bunching onion genomic DNA-derived SSRs/bulb onion EST-derived markers). On the other hand, we found a large number of SNPs from bulb onion EST-derived non-SSR markers between bunching onion and shallot through sequence analysis. Most of the SNP loci were successfully converted to CAPS markers. Although bunching onion and bulb onion belong to the section *Cepa* in the genus *Allium* and have the same number of chromosomes ($2n = 16$), bunching onion has a 28% smaller genome than bulb onion (Ricroch et al. 2005). Nevertheless, the large difference of nuclear DNA content is the result of an increase in the amount of repetitive sequences in bulb onion, suggesting that massive DNA modifications did not contribute to the speciation of these species (Narayan 1988). That is, a high level of genomic synteny might exist between bunching onion and bulb onion. This speculation has been supported by molecular marker analysis between these two species in a previous study (Tsukazaki et al. 2008).

Shallot–bunching onion monosomic addition lines have been used for both morphological and biochemical characterization (Shigyo et al. 1997), as well as chromosomal assignment of useful genes (Masuzaki et al. 2006a; McCallum et al. 2007; Yaguchi et al. 2008). Masuzaki et al. (2006b) also determined the chromosome identities of bulb onion SSRs developed by Fischer and Bachmann (2000) using these lines. However, these markers require intricate PCR cycling conditions, and appear to amplify several loci. Therefore, it is difficult to apply them to genetic studies such as comparing linkage maps with common markers. In contrast, recently developed bunching onion SSRs (Song et al. 2004; Tsukazaki et al. 2007) and bulb onion EST-derived SSRs (Kuhl et al. 2004; Martin et al. 2005) are more specific, and most of these markers produce single amplicons. By using these markers, we simply and readily determined the chromosomal locations of 406 markers using the *Allium* basic chromosomes and both shallot monosomic additions and bunching onion single alien deletions in the present study.

SSR markers for which chromosome identities were determined were shown to be highly polymorphic in bunching onion lines. The average PIC value (0.55) for 153 loci in 11 bunching onion lines was the same as in our previous studies (Song et al. 2004; Tsukazaki et al. 2007). Although bunching onion SSR markers were less polymorphic in bulb onion lines, some of these markers successfully segregated in the bulb onion F_2 population. No correlation ($r = -0.32$) was found between the average PIC value in bunching onion and bulb onion (data not shown). Hence, bunching onion SSRs will be useful as molecular markers in bulb onion, even if they were found to be monomorphic in bunching onion lines.

An SSR marker, AFAT06C11, which was determined to be located on chromosome 5C, was not polymorphic between parental lines of the SaT03 population. However, it was polymorphic between parental lines of the DJ population, and was successfully mapped to linkage group LGX of the DJ map (Fig. 1). We thus demonstrated that LGX was assigned to chromosome 5 (Chr.5) and denoted this region Chr.5b. All linkage groups of the DJ map have been completely assigned to the eight *A. fistulosum* chromosomes.

Monosomic addition lines are also useful for re-evaluation of the linkage map. In bulb onion, an AFLP-based map was constructed by van Heusden et al. (2000a). However, the authors noted discrepancies in the grouping of this map when assigning markers to the chromosomes using monosomic addition lines (van Heusden et al. 2000b). Another RFLP and EST-based bulb onion map also assigned linkage groups to bulb onion chromosomes using these lines (Martin et al. 2005). Nevertheless, we found that at least a part of the linkage group assigned to Chr.6 of the bulb onion map should instead be assigned to Chr.2 after comparison with the bunching onion DJ map (Tsukazaki et al. 2008). We constructed another bunching onion linkage map (SaT03 map) in this study (Fig. 2). This map was efficiently constructed by preferentially selecting markers assigned to the chromosomes in advance. Markers located on the linkage groups were in accordance with the assigned chromosomes.

On the other hand, several discrepancies were found by comparing this map to the DJ map (data not shown). For example, chromosomal locations of two bunching onion SSR markers, AFC12 and AFA06A10, on the SaT03 map (Chr.1 and Chr.2) were different from those on the DJ map (Chr.2 and Chr.3, Tsukazaki et al. 2008). We reanalyzed these markers for the DJ population, and the chromosomal locations of these markers in the rearranged DJ map were consistent with those in the SaT03 map (data not shown). Hence, AFC12 and AFA06A10 should be correctly located on Chr.1 and Chr.2, respectively (Fig. 2). Thus, comparison between several maps based on common markers is an effective method of improving the quality of linkage maps.

In conclusion, we succeeded in determining the chromosome identities for 766 markers. These markers were useful for the construction of an additional linkage map for a bunching onion population. In addition, these chromosome-specific markers will be useful for intensive mapping on specific chromosomes to obtain DNA markers linked to desirable QTLs for agricultural traits, as well as for

comparing linkage map-based genomic synteny between bunching onion and bulb onion.

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