African Journal of Biotechnology Vol. 11(11), pp. 2654-2660, 7 February, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.3307
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Development of simple sequence repeat (SSR) markers that are polymorphic between cultivars in *Brassica rapa* subsp. *rapa*

Weilin Wu[1,3], Bo Zhou[1], Dan Luo[1], Haifang Yan[1], Yuhua Li[1*] and Saneyuki Kawabata[2*]

[1]College of Life Sciences, Northeast Forestry University, Harbin 150040, China.
[2]Graduate School of Agricultural and Life Sciences, University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan.
[3]College of Agricultural Yanbian University Yanji,133000 China

Accepted 3 January, 2012

Simple sequence repeats (SSRs) markers were developed through data mining of 3,803 expressed sequence tags (ESTs) previously published. A total of 144 di- to penta-type SSRs were identified and they were screened for polymorphism between two turnip cultivars, 'Tsuda' and 'Yurugi Akamaru'. Out of 90 EST-SSRs for which polymerase chain reaction (PCR) primers were designed, 85 could be successfully amplified, 13 showed polymorphism and eight were regarded as valid markers based on Hardy-Weinberg equilibrium (HWE) in the F_2 population developed from a cross between the two cultivars. Additionally, 1551 SSR markers reported in public database were also subjected to polymorphism survey. Among these SSR markers, 66 showed polymorphism between the two cultivars, while 28 showed HWE with Ho in a range of 0.4 to 0.6. A linkage map was constructed using these eight EST-SST markers and 28 published SSR markers in the F_2 population.

Key words: EST-SSR, linkage map, polymorphism, single sequence repeats, turnip.

INTRODUCTION

The genus *Brassica* is a core genus of Brassicaceae family and includes various important crops worldwide. Based on allopolyploidy of the genome, the genus is grouped into three ancestral diploids, *Brassica rapa* (AA genome, 2n = 20), *Brassica nigra* (BB genome, 2n = 16) and *Brassica oleracea* (CC genome, 2n = 18), and three tetraploids between these parental diploids. Many economically important food crops belong to *B. rapa*. *B. oleracea* comprised of several subgroups, which present a large morphological divergence. Examples of *B. oleracea* include cabbage, kale, broccoli cauliflower, brussels sprouts, and kohlrabi, while *B*. *rapa* includes Chinese cabbage, pakchoi, and turnip.A genetic linkage map was constructed for *B. rapa* (Choi et al., 2007) using 278 amplified fragment length polymorphism (AFLP), 235 simple sequence repeats (SSRs), 25 random amplification of polymorphic DNA (RAPD) and 18 expressed sequence tag (EST) and sequence tagged site (STS or CAPS) markers between two Chinese cabbage (*B. rapa* subsp. *pekinensis*) inbred lines. For another *B. rapa* subspecies turnip (*B. rapa* subsp. *rapa*), however, there are still a limited number of DNA markers that may provide sufficient anchors for mapping inheritable traits on the genome. SSR is becoming increasingly recognized as useful DNA markers for genetic analysis, marker assisted selection, and other purposes (Gupta and Varshney, 2000). In addition, the released draft *B. rapa* genome sequence covers only 384 Mb of the 529 Mb Brassica A genome (Ramchiary et al., 2011).

ESTs are sequenced portions of complementary DNA copies of mRNA; they represent part of the transcribed portion of the genome in given conditions. As expected, they mainly correspond to relatively conserved sequences. These sequences from transcribed genes are Assembled into unique gene sequences and used to

*Corresponding authors. E-mail: liyuhua_nefu@126.com, ayuki@mail.ecc.u-tokyo.ac.jp.

Abbreviations: SSRs, Simple sequence repeats; **HWE,** Hardy-Weinberg equilibrium; **ESTs,** expressed sequence tags.

design SSRs from genes with a unique identity and position in the genome (Parida et al., 2006, 2010; Sharma et al., 2009). Therefore, an ever-increasing number of EST sequences provide a complementary source for microsatellite marker identification. Although the conserved nature of coding sequences may limit their polymorphism, it could facilitate cross-amplification of loci among phylogenetically related species (Scott et al., 2000) and even genera. Moreover, they have a high probability of being associated with functional portions of the genome. Among their many applications, these EST derived markers (EST-SSR) can be used to cross-reference genes between species for enhancing the resolution in comparative genomics studies and identifying conserved genomic regions among species and genera (Brown et al., 2001; Decroocq et al., 2003; Gupta et al., 2003; Saha et al., 2004; Yu et al., 2004; Park et al., 2005; Varshney et al., 2005).

As a byproduct of massive EST sequencing, it became possible to develop microsatellite-associated markers through data mining of such ESTs, as an inexpensive, time-saving, and effective approach in plants (Cordeiro et al., 2001; Kantety et al., 2002; Chen et al., 2006) and animals (Serapion et al., 2004; Yue et al., 2004; Chen et al., 2005; Pérez et al., 2005; Wang et al., 2005). In this study, we developed SSR markers polymorphic between two turnip cultivars by screening markers developed for *Brassica* species and by mining cDNA sequence data obtained previously for turnip.

MATERIALS AND METHODS

F_2 population comprising of 259 individuals was developed from a cross between *B. rapa* subsp. *rapa* 'Tsuda' that exhibited pigmentation only in the above ground portion of the hypocotyls resulting in bicolored (red and white) tissue, and 'Yurugi Akamaru' of which the entire swollen hypocotyls exhibit pigmentation. Genomic DNA was extracted from fresh leaves using hexadecyltrimethylammonium bromide (CTAB) method (Aljanabi and Martinez, 1997).

Data mining, polymerase chain reaction (PCR) amplification and detection of SSR

A total of 3,803 EST sequences obtained from cDNA libraries constructed for 'Tsuda' turnip (Zhou et al., 2007) were subjected to screening for SSRs containing at least four tandem repeating units of di-, tri-, tetra-, penta- and hexa-nucleotide by using 'SSR Hunter 1.3' (Li and Wan, 2005). PCR primers for amplifying SSR region were designed on a web application 'Primer 3' setting parameters as: annealing temperature of 55 to 65°C and expected product size of 100 to 300 bp. One 'best' primer pair was tested for SSR marker detection. These primer pairs as well as published 1551 primer pairs developed for *B. rapa, B. oleracea, B. napus* and *B. nigra* were subjected to polymorphism detection.

SSR region was amplified on a thermal cycler (PTC-100, MJ Research) using the program: 94°C for 5 min, 34 cycles of 94°C for 30 s, annealing temperatures shown in Table 1 for 30 s, 72°C for 45 s, and a final extension at 72°C for 7 min. The PCR was performed in a 20-μL reaction mixture, containing 1×PCR buffer, 20 ng sample DNA, 5.0 μM of each primer, 1.8 mM $MgCl_2$, 1.0 μM of each dNTP and 1.0 U of Taq DNA polymerase. PCR products were separated by electrophoresis on a 12% non-denaturing polyacrylamide gel electrophoresis in 1×TBE buffer and visualized by silver-staining (Zhang and Bai, 2008). To confirm amplification of SSRs, DNA fragments were excised from the gel and purified using the EZ-10 Spin Column DNA Gel Extraction Kit (Bibasic Inc.), and sequenced after cloning into a pUC18 vector.

Construction of linkage maps

For all polymorphic SSR markers, observed (Ho), expected (He) heterozygosity, and the fitness to the Hardy-Weinberg equilibrium (HWE) were calculated using a software 'POPGENE version 1.3.1' (Yeh et al., 1999). When the H_O was in a range of 0.4 to 0.6 and P value of HWE deviation was at >0.05, the markers is regarded valid polymorphic marker. Linkage analysis was conducted using AntMap Ver. 1.2 (Iwata and Ninomiya, 2006) and the map was drawn using MapChart (Voorrips, 2002). Linked loci were grouped based on maximum recombination fraction of 0.3. Grouped SSR marker loci were evaluated for the reliability of the estimated locus order by a bootstrap test. Map distances (cM) were calculated using the Kosambi mapping function. Some of markers were mapped on a *B. rapa* genomic database (chromosome ver1.1, http:// brassicadb.org/brad/) and compared with the linkage map.

RESULTS

Development of EST-SSR markers

From the EST data set, SSRs were found in 144 ESTs. The calculated frequency of SSR was 3.8% of screened ESTs and one for every 9.54 kb. Most of these EST-SSRs were composed of dinucleotide and trinucleotide repeats. The proportions of di-, tri-, tetra- and penta-nucleotide motifs among these ESTs were 67.1, 30.8, 2.1 and 0.7%, respectively. The AG/CT di-repeats and AGA/TCT or ATG/CAT tri-repeats accounted for one fourth of total EST-SSRs. The CG repeats was the least di-repeats. Among the tri-repeats, AT-rich types were predominant, while GC-rich types were minor.

Out of 85 EST-SSR markers in which PCR primer pairs could be designed for successful amplification, 13 (14.4%) showed polymorphism between the two cultivars tested. All the amplified DNA fragments for these 13 were sequenced to confirm valid SSR markers with their flanking sequences showing a high homology, and to also see repeat numbers. The Ho of these candidate EST-SSR markers within the F_2 population ranged from 0.156 to 0.683, and He ranged from 0.031 to 0.500. Eight EST-SSRs were regarded as valid markers based on HWE.

Polymorphism analysis of published SSR markers

Among the 1,551 published SSR markers tested, only 66 of them (4.26%) showed polymorphism in F_2 population. Based on HWE and Ho which was in a range of 0.4 to

Table 1. List of 13 polymorphic EST-SSR markers developed between 'Tsuda' and 'Yurugi Akamaru' turnip.

Locus	Motif	Primer 1 (5'-3')	Primer 2 (5'-3')	Ta (°C)	Number of allele	Amplified fragment sizes (bp)	Ho	He	P
BrEST-09	$(TC)_{5-15}$	AGCCTTCTCCCTGTTCTTGG	TTGGGTTCCTTGTCTCTGCT	57	2	162, 174	0.4556	0.4967	0.1728
BrEST-11	$(CTT)_{4-10}$	GCTCAAATGCGTTCATCATCT	ATGGGTAAGGTTCACGGTTC	57	2	629, 716	0.1367	0.2501	0.0000
BrEST-13	$(GTG)_{5-8}$	AGTCTGCTTCTGCTCCGTCT	CCTCCTTGACCACCACCAC	62	2	216, 225	0.5292	0.4999	0.3645
BrEST-14	$(GAT)_{6-9}$	GCCAAGGCTAATTCTAACTCCA	CAATCCAACTCGTTTTGACC	57	2	109, 118	0.5212	0.5000	0.5135
BrEST-24	$(CTC)_{4-7}$	CTCGCTCCTCTCCTTCTCCT	CTAAGCCCCATAGCTTTCTC	62	2	108, 117	0.5078	0.4994	0.8121
BrEST-26	$(GGA)_{4-10}$	CTAAGCCCCATAGCTTTCTC	CTCGCTCCTCTCCTTCTCCT	57	2	118, 136	0.5019	0.4994	0.9597
BrEST-33	$(GA)_{5-11}$	CGGCGATAGGAGAGAGAGG	GTAAGGACGCTCACGGTTT	60	2	162, 174	0.5020	0.4959	0.8707
BrEST-38	$(AGA)_{5-12}$	CGATCCAACAGCTCCTCTTC	CGTGGTTGACGACTTGAGAA	59	2	280, 351	0.4170	0.4875	0.0184
BrEST-56	$(AC)_{4-8}$	ATGGGTAAGGTTCACGGTTC	CTCCAATGCGTTCATCATCT	53	2	656, 693	0.2008	0.2971	0.0000
BrEST-62	$(CGA)_{5-13}$	ATGGCGACTTCAGATTCGAG	CCTCCTCCTCCTCATCATCA	57	2	155, 237	0.4196	0.4911	0.0184
BrEST-64	$(CA)_{8-13}$	AGGTCACACACACACACACAAA	GGAGCAGACTCGGTCACAAC	61	2	462, 472	0.4131	0.4474	0.2060
BrEST-85	$(CTC)_{4-7}$	ATCATTATCCCATCTCGCTCCT	GGACTTGGTCATGTGCTGTTT	62	2	100, 109	0.4961	0.4992	0.8952
BrEST-86	$(TGA)_{5-12}$	GAGTTTCAATCGGTGGCTCT	TCTCTTTGGGCTTCTTGGTG	53	2	378, 450	0.3436	0.4569	0.0001

Ta, Annealing temperature; Ho, observed heterozygosity; He, Nei's expected heterozygosity (Nei et al., 1973); P, probability in Hardy-Weinberg equilibrium.

0.6, a total of 28 remained as valid SSR markers.

Linkage analysis

A linkage map (Figure 1) was constructed using eight EST-SSR markers and 28 SSR markers with a recombination threshold of 0.3 and a minimum number of markers per group as 2. Total genetic lengths of the linkage maps were 341.4 cM. The largest linkage group has a length of 90.3 cM and comprised of seven markers.

Out of 36 markers, nine were not mapped to any linkage groups.

Comparison with *B. rapa* map showed that these linkage groups corresponded to chromosomes A01, A03, A08, A09, and A10, while two linkage groups could not be assigned to any chromosomes.

DISCUSSION

SSRs are the DNA regions where a short sequence unit tandemly repeated. They are ubiquitous in eukaryotes and prokaryotes and can be found both in the non-coding and coding region. In this study, we developed SSR markers using EST data set from *B. rapa* subsp. *rapa* (Zhou et al., 2007). Among the EST sequences analyzed, 3.8% contained SSRs. Among dicots species, the frequency of ESTs containing SSRs was found to range from 2.65 to 10.62% (Kumpatla and Mukhopadhyay, 2005). In the developed EST-SSRs, di-nucleotide repeating SSR was the most abundant, followed by tri-nucleotide repeats. Whereas di-nucleotides were the primary SSR repeats in Rosaceae ESTs (Jung et al., 2005) and were tri-nucleotides primary in *Arabidopsis* (Cardle et al., 2000). In all of these species, tetra- and penta-nucleotide repeats accounted for less than 5%. Among di-nucleotide repeats, AG/CT repeat was the most abundant, and CG/CG was minor as EST-SSR of other plants (Gupta and Varshney, 2000; Kantety et al., 2002; Han et al., 2004; Poncet et al., 2006) and vertebrates (Neff and Gross, 2001). In genomic sequences of *Arabidopsis*, the most common di-nucleotide repeats were AT/AT (Tóth et al., 2000). The most abundant tri-nucleotide repeats were AGA/TCT and ATG/CAT (25%), followed by CCA/TGG and AGG/CCT (18.18%) in turnip. These repeats are also major repeats in other dicots (Scott et al., 2000; Eujayl et al., 2004; Kumpatla and Mukhopadhyay, 2005).

It has been suggested that SSR length polymorphism of EST is generally lower than that of genomic DNA (Cho et al., 2000; Eujayl et al., 2001, 2002; Gupta et al., 2003). This is due to a

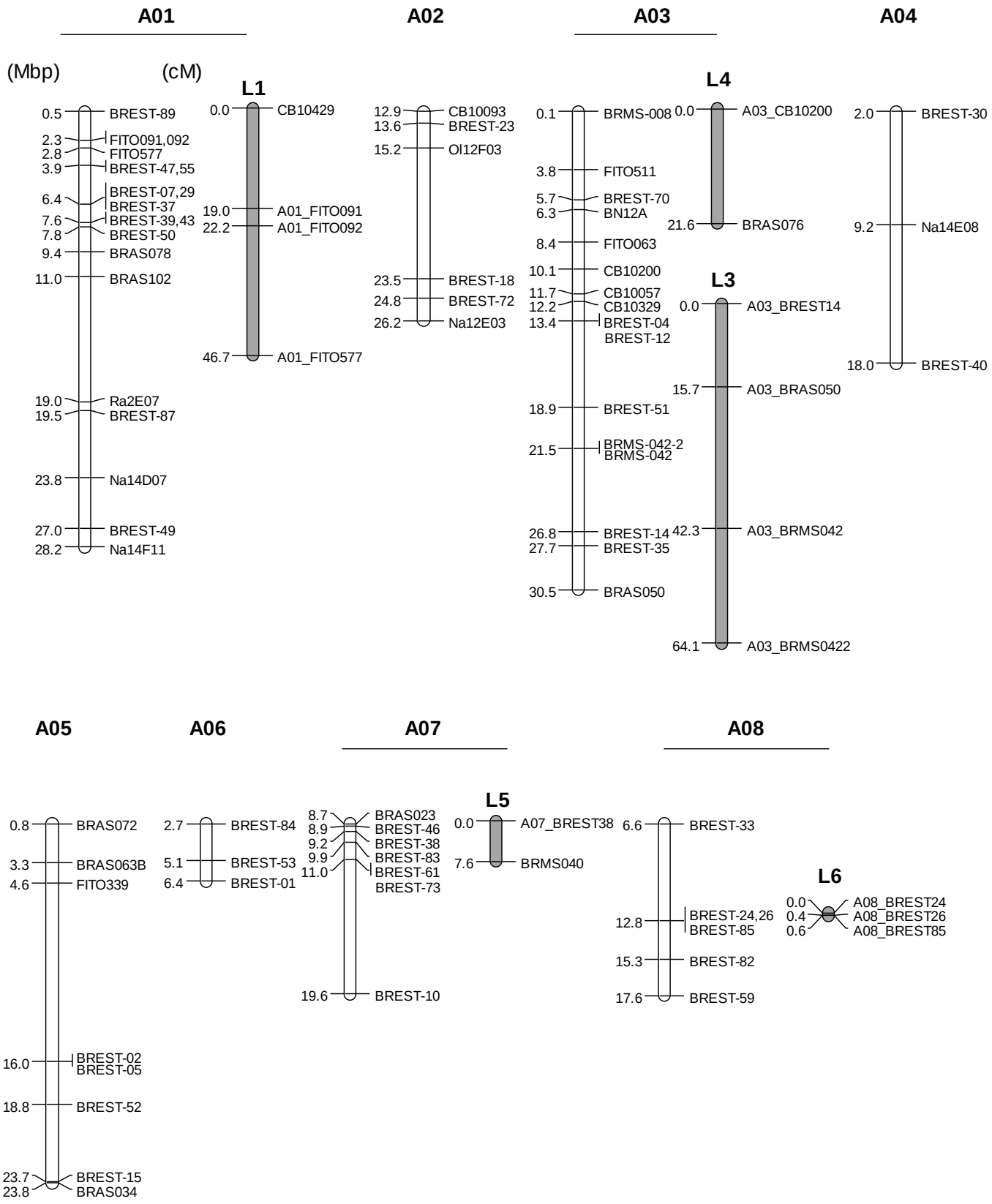

Figure 1. The linkage map of *Brassica rapa* subsp. *rapa* constructed based on 259 F_2 plants derived from a cross between 'Tsuda' and the 'Yurugi Akamaru' (grey bars, L01 – L08). The map of DNA markers on the A genome chromosomes of *B. rapa* subsp. *pekinensis* are determined using chromosome version 1.0 database and presented as white bars (A01 to A09).

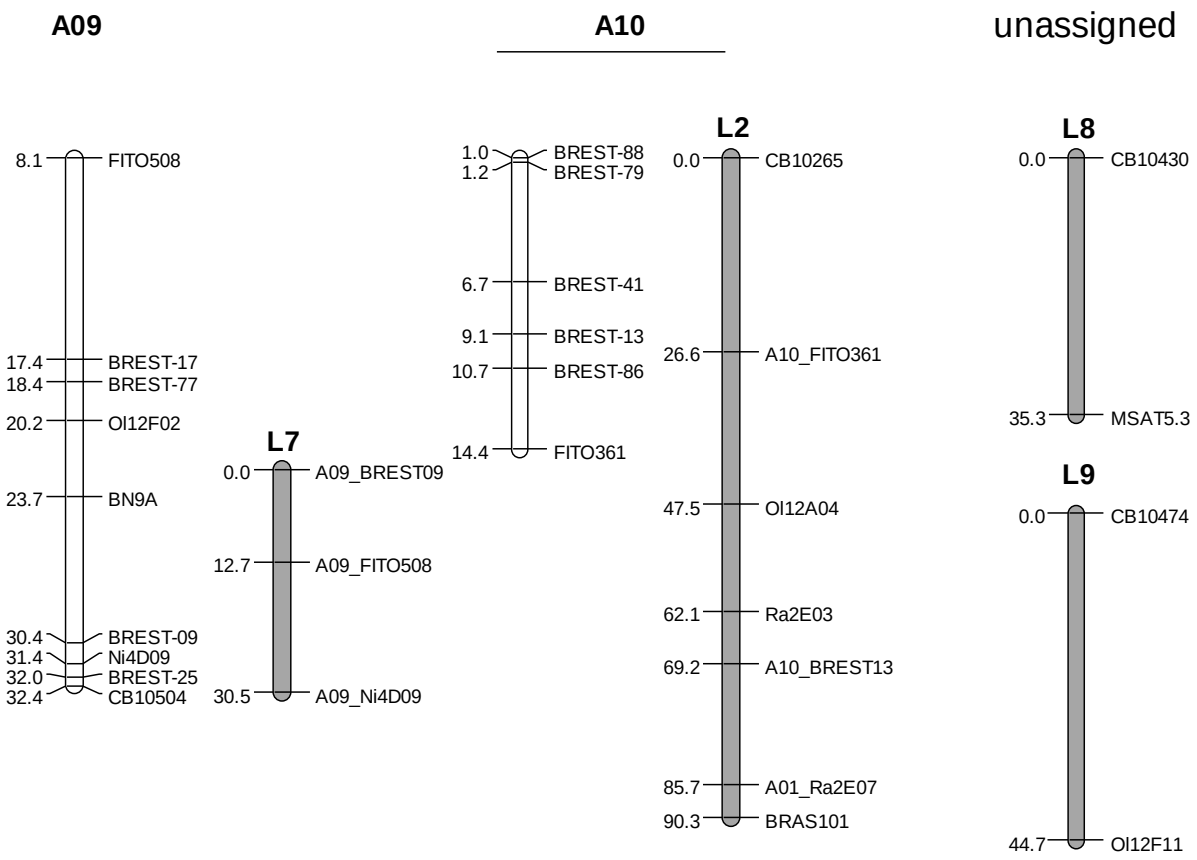

Figure 1. Contd.

lower mutation rate within gene-coding regions than non-coding regions. Nevertheless, some studies reported high levels (Fraser et al., 2004; Saha et al., 2004; Jung et al., 2005) or even higher levels (Varshney et al., 2005) of polymorphism of EST-SSR markers. The amplified products were often longer than expected length based on EST sequences. This result indicates the presence of introns in the amplified genomic DNA region (Cordeiro et al., 2001). As in other *Brassica* mapping studies, we found many loci showing deviations from the expected Mendelian ratios in F_2 populations. Such segregation distortion was also found in *Brassic*a (Slocum et al., 1990; Chyi et al., 1992; Teutonico and Osborn, 1994) and other plants such as *Lentil* (Havey and Muehlbauer, 1989) and tomato (Paterson et al., 1990).

This could be due to the occurrence of gametic or zygotic selection whereby certain gametes or zygotic combinations failed to develop after chromosomal rearrangement, or as a result of unintentional selection.

Conclusion

In this study, we screened EST-SSR and SSR markers polymorphic between two turnip cultivars by screening approximately 1,636 SSR marker candidates. We could develop eight new EST-SSR markers and 28 SSRs (Table 2). Although microsatellite markers such as SSR are one of the best sources for developing DNA markers, we could not develop sufficient number of SSRs for providing a reliable and effective means for genomics analysis.

Low frequency of valid polymorphic maker indicates that genetic diversity among turnip cultivars is low in *Brassica* species. To develop more markers sufficient for mapping useful traits, more advanced marker searches such as single nucleotide polymorphism (SNP) discovery by massive genomic sequencing would be required.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (30800082, 30730078), the Specialized Research Fund for the Doctoral Program of Higher Education (200802251039), the National Science Foundation for Post-doctoral Scientists of China (Grant No.20090460868, 201003409), and the Young Top-notch Talent support program of Northeast Forestry University (YTTP-1011-17).

Table 2. List of 28 published SSR markers which showed polymorphism between 'Tsuda' and 'Yurugi Akamaru' turnip.

Locus	Ho	He	P	Reference
BRAS050	0.514	0.498	0.611	Piquemal et al., 2005; Radoev et al., 2008
BRAS076	0.479	0.495	0.604	Piquemal et al., 2005; Radoev et al., 2008
BRAS101	0.467	0.493	0.401	Piquemal et al., 2005; Radoev et al., 2008
BRMS040	0.440	0.486	0.127	Suwabe et al., 2002
BRMS042	0.502	0.497	0.876	Suwabe et al., 2002
BRMS042-2	0.456	0.473	0.549	Suwabe et al., 2002
CB10093	0.533	0.499	0.272	Piquemal et al., 2005; Radoev et al., 2008
CB10200	0.502	0.493	0.765	Piquemal et al., 2005; Radoev et al., 2008
CB10265	0.529	0.499	0.334	Piquemal et al., 2005; Radoev et al., 2008
CB10429	0.494	0.489	0.865	Piquemal et al., 2005; Radoev et al., 2008
CB10430	0.475	0.486	0.723	Piquemal et al., 2005; Radoev et al., 2008
CB10474	0.429	0.470	0.152	Piquemal et al., 2005; Radoev et al., 2008
FITO091	0.498	0.499	0.975	Iniguez-Luy et al., 2009
FITO092	0.502	0.500	0.943	Iniguez-Luy et al., 2009
FITO339	0.521	0.501	0.504	Iniguez-Luy et al., 2009
FITO361	0.533	0.499	0.272	Iniguez-Luy et al., 2009
FITO508	0.463	0.484	0.489	Iniguez-Luy et al., 2009
FITO577	0.483	0.496	0.657	Iniguez-Luy et al., 2009
MSAT5.3	0.479	0.490	0.709	http://www.inra.fr/qtlat/msat/index.php
Na12E03	0.413	0.460	0.100	Lagercrantz et al., 1993; Lowe et al., 2002, 2004
Ni4D09	0.444	0.470	0.366	Lagercrantz et al., 1993; Lowe et al., 2002, 2004
Ol12A04	0.525	0.501	0.436	Lagercrantz et al., 1993; Lowe et al., 2002, 2004
Ol12F02	0.463	0.497	0.274	Lagercrantz et al., 1993; Lowe et al., 2002, 2004
Ol12F03	0.533	0.501	0.305	Lagercrantz et al., 1993; Lowe et al., 2002, 2004
Ol12F11	0.429	0.476	0.106	Lagercrantz et al., 1993; Lowe et al., 2002, 2004
Ra2C09	0.498	0.501	0.927	Lagercrantz et al., 1993; Lowe et al., 2002, 2004
Ra2E03	0.537	0.500	0.235	Lagercrantz et al., 1993; Lowe et al., 2002, 2004
Ra2E07	0.502	0.498	0.898	Lagercrantz et al., 1993; Lowe et al., 2002, 2004

Ho, Observed heterozygosity; He, Nei's expected heterozygosity (Nei et al., 1973); P, probability in Hardy-Weinberg equilibrium.

REFERENCES

Aljanabi SM, Martinez I (1997). Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. Nuc. Acids Res. 25: 4692-4693.

Brown GR, Kadel EE III, Bassoni DL, Kiehne KL, Temesgen B, van Buijtenen JP, Sewell MM, Marshall KA, Neale DB (2001). Anchored reference loci in loblolly pine (*Pinus taeda L*.) for integrating pine genomics. Genetics 159: 799-809

Cardle L, Ramsay L, Milbourne D, Macaulay M, Marshall D, Waugh R (2000). Computational and experimental characterization of physically clustered simple sequence repeats in plants. Genet. 56: 847-854.

Chen SL, Liu YG, Xu MY, Li J (2005). Isolation and characterization of polymorphic microsatellite loci from an EST-library of red sea bream (*Chrysophrys major*) and cross-species amplification. Mol. Ecol. Notes 5: 215-217.

Chen C, Zhou P, Choi YA, Huang S, Gmitter FG (2006). Mining and characterizing microsatellites from citrus ESTs. Theor. Appl. Genet. 112: 1248-1257.

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.

Choi SR, Teakle GR, Plaha P, Kim JH, Allender CJ, Beynon E, Piao ZY, Soengas P, Han TH, King GJ, Barker GC, Hand P, Lydiate DJ, Batley J, Edwards D, Koo DH, Bang JW, Park BS, Lim YP (2007). The reference genetic linkage map for the multinational *Brassica rapa* genome sequencing project. Theor. Appl. Genet. 115: 777-792.

Chyi YS, Hoenecke ME, Sernyk JL (1992). A genetic linkage map of restriction fragment length polymorphism loci for *Brassica rapa* (syn. *campestris*). Genome 35: 746-757.

Cordeiro GM, Casu R, McIntyre CL, Manners JM, Henry RJ (2001). Microsatellite markers from sugarcane (*Saccharum spp.*) ESTs cross transferable to erianthus and sorghum. Plant Sci. 160: 1115-1123.

Decroocq V, Fave MG, Hagen L, Bordenave L, Decroocq S (2003). Development and transferability of apricot and grape EST microsatellite markers across taxa. Theor. Appl. Genet. 106: 912-922

Eujayl I, Sorrells M, Baum M, Wolters P, Powell W (2001). Assessment of genotypic variation among cultivated durum wheat based on EST-

SSRs and genomic SSRs. Euphytica, 119: 39-43.
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.
Eujayl I, Sledge MK, Wang L, May GD, Chekhovskiy K, Zwonitzer JC, Mian MAR (2004). *Medicago truncatula* EST-SSRs reveal cross-species genetic markers for *Medicago* spp. Theor. Appl. Genet. 108: 414-422.
Fraser LG, Harvey CF, Crowhurst RN, De Silva HN (2004). EST-derived microsatellites from *Actinidia* species and their potential for mapping. Theor. Appl. Genet. 108: 1010-1016.
Gupta PK, Varshney RK (2000). The development of and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. Euphytica 113: 163-185.
Gupta PK, Rustgi S, Sharma S, Singh R, Kumar N, Balyan HS (2003). Transferable EST-SSR markers for the study of polymorphism and genetic diversity in bread wheat. Mol. Gen. Genomics, 270: 315-323.
Han ZG, Guo WZ, Song XL, Zhang TZ (2004). Genetic mapping of EST-derived microsatellites from the diploid *Gossypium arboretum* in allotetraploid cotton. Mol. Gen. Genomics, 272: 308-327.
Havey MJ, Muehlbauer FJ (1989). Linkages between restriction fragment length, isozyme, and morphological markers in lentil. Theor. Appl. Genet. 77: 395-401.
Iwata H, Ninomiya S (2006). AntMap: Constructing Genetic Linkage Maps Using an Ant Colony Optimization Algorithm. Breeding Sci. 56: 371-377.
Jung S, Abbott A, Jesudurai C, Tomkins J, Dorrie M (2005). Frequency, type, distribution and annotation of simple sequence repeats in Rosaceae ESTs. Funct. Integr. Genomics, 5: 136-143.
Kantety RV, Rota ML, Matthews DE, Sorrells ME (2002). Data mining for simple sequence repeats in expressed sequence tags from barley, maize, rice, sorghum and wheat. Plant Mol. Biol. 48: 501-510.
Kumpatla SP, Mukhopadhyay S (2005). Mining and survey of simple sequence repeats in expressed sequence tags of dicotyledonous species. Genome, 48: 985-998.
Li Q, Wan JM (2005). SSRHunter: Development of a local searching software for SSR sites. Hereditas, 27: 808-810.
Neff BD, Gross MR (2001). Microsatellite evolution in vertebrates: inference from AC dinucleotide repeats. Evolut. 55: 1717-1733.
Parida SK, Anand Raj Kumar K, Dalal V, Singh NK and Mohapatra T (2006). Unigene derived microsatellite markers for the cereal genomes. Theor. Appl. Genet. 112: 808-817.
Parida SK, Yadava DK Mohapatra T (2010). Microsatellites in Brassica unigenes: relative abundance,marker design, and use in comparative physical mapping and genome analysis. Genome 53: 55-67.
Park YH, Alabady MS, Ulloa M, Sickler B, Wilkins TA, Yu J, Stelly DM, Kohel RJ, el-Shihy OM, Cantrell RG (2005). Genetic mapping of new cotton fiber loci using EST-derived microsatellites in an interspecific recombinant inbred line cotton population. Mol. Genet. Genomics, 274: 428-441
Paterson AH, Deverna JW, Lanini B, Tanksley SD (1990). Fine mapping of quantitative trait loci using selected overlapping recombinant chromosomes, in an interspecies cross of tomato. Genetics, 124: 735-742.
Pérez F, Ortiz J, Zhinaula M, Gonzabay C, Calderón J, Volckaert FAMJ (2005). Development of EST-SSR markers by data mining in three species of shrimp: *Litopenaeus vannamei*, *Litopenaeus stylirostris*, and *Trachypenaeus birdy*. Mar. Biotechnol. 7: 554-569.
Poncet V, Rondeau M, Tranchant C, Cayrel A, Hamon S, Kochko AD, Hamon P (2006). SSR mining in coffee tree EST databases: potential use of EST-SSRs as markers for the *Coffea* genus. Mol. Gen. Genomics, 276: 436-449.
Ramchiary N, Nguyen DV, Li XN, Hong CP, Dhandapani V, Choi SR, Ge Y, Piao ZY, Lim YP (2011). Genic microsatellite markers in *Brassica rapa*: development, characterisation, mapping, and their utility in other cultivated and wild *Brassica* relatives. DNA Res.18: 305-320
Saha MC, Rouf Mian MA, Eujayl I, Zwonitzer JC, Wang L, May GD (2004). Tall fescue EST-SSR markers with transferability across several grass species. Theor. Appl. Genet. 109: 783-791.
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.
Serapion J, Kucuktas H, Feng J, Liu Z (2004). Bioinformatic mining of type I microsatellites from expressed sequence tags of channel catfish (*Ictalurus punctatus*). Mar. Biotechnol. 6: 364-377.
Sharma RK, Bhardwaj P, Negi R, Mohapatra T, Ahuja PS (2009). Identification, characterization and utilization of unigene derived microsatellite markers in tea (*Camellia sinensis L*.). BMC Plant Biol. 9: p. 53.
Slocum MK, Figdore SS, Kennard WC, Suzuki JY, Osborn TC (1990). Linkage arrangement of restriction fragment length polymorphism loci in *Brassica oleracea*. Theor. Appl. Genet. 80: 57-64.
Teutonico RA, Osborn TC (1994). Mapping of RFLP and qualitative trait loci in *Brassica rapa* and comparison to the linkage maps of *B. napus*, *B. oleracea*, and *Arabidopsis thaliana*. Theor. Appl. Genet. 89: 885-894.
Tóth G, Gáspári Z, Jurka J (2000). Microsatellites in different eukaryotic genomes: survey and analysis. Genome Res. 10: 967-981.
Varshney RK, Graner A, Sorrells ME (2005). Genic microsatellite markers in plants: features and applications. Trends Biotechnol. 23: 48-55.
Varshney RK, Sigmund R, Borner A, Korzun V, Stein N, Sorrells ME, Langridge P, Graner A (2005). Interspecific transferability and comparative mapping of barley EST-SSR markers in wheat, rye and rice. Plant Sci. 168: 195-202
Voorrips RE (2002). MapChart: Software for the graphical presentation of linkage maps and QTLs. J. Heredity, 93: 77-78.
Wang H, Li F, Xiang J (2005). Polymorphic EST-SSR markers and their mode of inheritance in *Fenneropenaeus chinensis*. Aquaculture, 249: 107-114.
Yeh FC, Yang RC, Boyle T (1999). POPGENE, Version 1.3.1 [www.ualberta.ca/~fyeh]
Yu JK, La Rota M, Kantety RV, Sorrells ME (2004). EST derived SSR markers for comparative mapping in wheat and rice. Mol. Genet. Genomics, 271: 742-751
Yue GH, Ho MY, Orban L, Komen J (2004). Microsatellites within genes and ESTs of common carp and their applicability in silver crucian carp. Aquaculture, 234: 85-98.
Zhang YS, Bai XF (2008). A simple, rapid and high-resolution banding method in polyacrylamide gels. Hereditas, 30: 251-254.
Zhou B, Li YH, Xu ZR, Yan HF, Honma S, Kawabata S (2007). Ultraviolet A-specific induction of anthocyanin biosynthesis in the swollen hypocotyls of turnip (*Brassica rapa*). J. Exp. Bot. 58: 1771-178.