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# Isolation of New Microsatellite-containing Sequences in *Acanthopanax senticosus*

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**Microsatellite markers, also called simple sequence repeats (SSRs), are comprised of a 2- to 6-nucleotide repeat motif. They are useful as molecular markers for genetic authentication, crop breeding programs, and linkage analysis for map-based cloning. From a microsatellite-enriched genomic library of *Acanthopanax senticosus*, we identified 239 new microsatellite-containing sequences. The di-nucleotide repeat units were the most abundant (55.2%), followed by tri-nucleotide repeat units (24.6%). In detailed repeat structures, the $(AG)_n$ motif was most frequent (30.5%), followed by the $(AC)_n$ motif (21.7%). Hepta- and octa-nucleotide repeat motifs were found in each single locus, and a total of 33 (13.8%) complex repeat structures were recorded. This is the first report of mass isolation of microsatellites via screening of an *A. senticosus* library, and may well provide information useful as a genetic resource for the further study of *A. senticosus*.**

*Keywords*: *Acanthopanax senticosus*, genetic marker, microsatellites, simple sequence repeats (SSRs)

*Acanthopanax senticosus,* also called "Siberian ginseng" (syn. *Eleutherococcus senticosus*), has long been used in Asia as a traditional medical remedy (Balasubramaniyan et al., 2003). Its main phytochemical components are eleutherosides A through G. Eleutherosides B (syringin) and E (liriodendrin) are known as the active components (Awang, 1996), and reportedly have several therapeutic effects, e.g., anti-fatigue, anti-stress, immuno-enhancement, and anti-depression.

Numerous investigations have been conducted toward the development of molecular makers as genetic resources. Techniques have included restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD) (Cho et al., 1996; Mihalov et al., 2000; Um et al., 2001), amplified fragment length polymorphism (AFLP), and directed amplification of minisatellite region DNA (Ha et al., 2002). Although these approaches have been successful, they have limitations at several points, e.g., low levels of polymorphism detection and/or reproducibility, and the need for a considerable amount of sample.

Microsatellites, i.e., simple sequence repeats (SSRs), consist of the tandem repeat of 2 to 6 nucleotides (nt). Because of their extensive variability and broad genomic distribution (Tautz, 1989), they have been widely used for map-based cloning, the construction of genome-wide physical maps, and furthering our understanding of genome evolution (Bonierbale et al., 1988; Martin et al., 1993; Tanksley et al., 1996; Kurata et al., 1997; Bao et al., 2006). Microsatellites possess such advantageous traits as co-dominance and multiallelism. Likewise, their high informative content and distribution enable their applications for authentication and linkage analysis-related species, making them important tools that avoid the problems of lower sensitivity or reproducibility typical of other approaches. In particular, because most repeat motifs have a highly conserved flanking sequence, they can be used for identifying inter- or intra-specific relationships (Roder et al., 1995; Chambers and MacAvoy, 2000).

In the distribution of microsatellites, different patterns in composition are apparent between the coding and non-coding regions (Metzgar et al., 2000). Tri- and hexa- repeats are more numerous in the coding region while other repeat motifs are commonly observed in the non-coding region. This suggests that both regions must be screened if we are to improve our understanding of the actual composition of microsatellites across the entire genome.

In this study, we constructed a microsatellite-enriched *A. senticosus* genomic library and isolated 239 new microsatellite-containing sequences. Each microsatellite was classified according to the structure of a repeat unit. Our objective for this mass isolation was to increase the availability of genetic resources for this species.

## MATERIALS AND METHODS

### Plant Materials and DNA Extraction

Leaf tissues of *A. senticosus* were obtained from a herbal plant farm near the Gongju. Genomic DNA was extracted with a DNeasy plant isolation kit (Qiagen, Germany), according to the manufacturer's instructions (Fig. 1A).

### Preparation of a Microsatellite-enriched Library

A microsatellite-enriched library was constructed using a protocol modified from that of Hamilton et al. (1999) and Fischer and Bachmann (1998). Genomic DNA was digested with the *Sau3*AI restriction enzyme (Fig. 1B) and ligated with a *Sau3*AI-specific adaptor, the hybrid of oligonucleotides A and B (Oligo-A: 5′-GGCCAGAGACCCCAAGCTTCG-3′; Oligo-B: 5′-GATCCGAAGCTTGGGGTCTCTGGCC-3′). The adaptor-ligated DNA fragments ranged of 0.2 to 1.0 kb were eluted from 2% agarose gel (Fig. 1C), and then hybridized with 12 kinds of 5′-biotinilated oligonucleotides that represented different repetitive sequences (Table 1). Hybridization was performed in a 50-μL final volume reaction mixture containing size-fractionated DNA, 100 μM of bioti-

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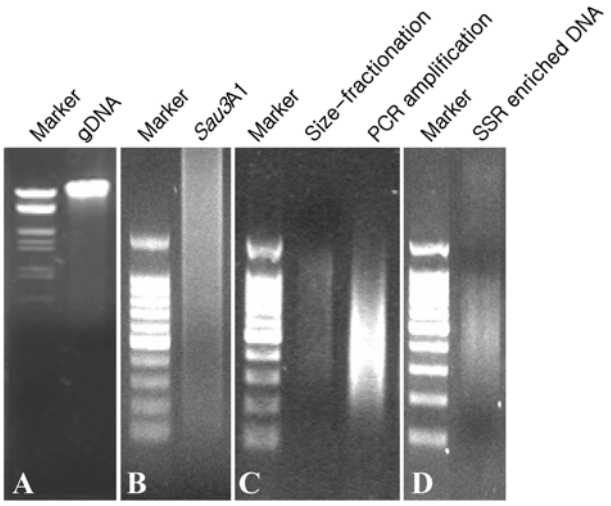


**Figure 1.** Construction of microsatellite enriched genomic library. (A) Genomic DNA from *A. senticosus*. Marker: lambda DNA/*Eco*RI + *Hind*III. (B) *Sau3*AI digestion of genomic DNA. *A. secticosus* genomic DNA was digested with *Sau3*A1 for 3 h and visualized in 2% agarose gel. Marker: 100 bp ladder. (C) Adaptor ligation and size-fractionation. DNA fragments were ligated with *Sau3*A1 adaptor, and fragments ranged of 0.2 to 1 kb were eluted from agarose gel. PCR was performed with the oligo A as the primer to confirm the adaptor ligation efficiency. Marker: 100 bp ladder. (D) Capture of microsatellite-containing DNA fragments. After hybridization with biotin-oligonucleotides, the microsatellite-containing sequences were captured using PMP-SA particles. Marker: 100 bp ladder.

**Table 1.** Biotinilated oligonucleotides used in the preparation of a microsatellite-enriched library from *A. senticosus*.

| Repeat unit | Oligomer sequences (Biotin-5′ → 3′) |
|---|---|
| Di-nt repeats | $(AT)_{15}$, $(AC)_{15}$, $(AG)_{15}$ |
| Tri-nt repeats | $(ACC)_{10}$, $(TAA)_{10}$, $(CAA)_{10}$, $(GAA)_{10}$, $(ACG)_{10}$ |
| Tetra-nt repeats | $(AAAG)_7$, $(AGAT)_7$, $(AAAT)_7$, $(ACAT)_7$ |

nilated oligonucleotides, and a 2X hybridization solution (12X SSC, 0.2% SDS), using a thermal cycler. The process included incubation at 95°C for 10 min, then 75 for 1 min, and 70 to 50.2°C touchdown (–0.2°C over 10 s), followed by another 50 to 40°C touchdown (–0.5°C over 10 s). After hybridization, and to enhance the capture rate, the hybrid mixture was incubated for 2 h with gentle shaking at 33°C with magnetic beads coated by streptavidin (Promega, USA). The beads were then washed twice with low stringency buffer (6X SSC, 0.1% SDS) at room temperature, twice with high stringency buffer (1X SSC, 0.1% SDS) at 40°C, and twice with the same (latter) buffer solution but at 50°C. The microsatellite-containing sequences were then eluted from the magnetic beads by applying heat (95°C for 2 min) to a Tris buffer (10 mM Tris, pH 7.5). Afterward, the eluted DNA fragments were once amplified by the PCR method with the oligo A as the primer (Fig. 1D), and were cloned into the pGEM-T easy vector (Promega, USA), which were also used transform *E. coli* (*DH10B*).

### Library Screen

Transformants were selected on LB plates containing ampicillin (50 mg/mL), X-gal (20 mg/mL), and IPTG (200 mg/mL). White colonies were sub-cultured in LB liquid media containing ampicillin (50 mg/mL), and plasmid DNA was isolated from the cultures using a plasmid mini-prep kit (SolGent, Korea). Each insert DNA was confirmed by performing *Eco*RI digestion of plasmid DNA, and the digestion patterns were visualized in a 1.5% agarose gel for size determination. The sequencing of insert DNA was carried out by using an ABI 3100 automatic sequencing analyzer (Applied Biosystems, USA) with universal primers T7 and SP6.

## RESULTS

### Construction of an *A. senticosus* Microsatellite-enriched Library

Twelve kinds of 2- to 4-nt simple repeat biotinilated oligonucleotides (Table 1) were used to capture the microsatellite-containing DNA fragments, thus enabling our construction of an enriched library. DNA fragments isolated from magnetic beads were sub-cloned into the pGEM-T easy vector. Transformation efficiency was about $5 \times 10^5$, with insert sizes ranging from approximately 0.2 to 1.2 kb. From the *Eco*RI digestion of 1303 randomly selected white clones, 1190 (91.3%) were verified as having been inserted, and were analyzed by sequencing without performing the usual step of colony hybridization.

We used the T7 and SP6 universal primers for this sequence analysis and found that approximately 33.9% (403) of the 1190 clones with inserts had microsatellite-containing sequences. We classified a microsatellite as a genomic sequence showing either a 2-nt sequence with a repeat number of five or more, or a 3- to 6-nt sequence with a repeat number of at least four.

### Characterization of Microsatellite-containing Sequences

We identified 403 microsatellite-containing sequences; all were classified according to their repeat unit, and the same repeat units were compared with each other using sequence alignments to remove the same clone. Consequently, we found that a considerable number of clones overlapped completely or partially. Subsequently, 239 independent microsatellite-containing sequences were determined from this library screening (59.3%, 239/403).

The observed frequencies of di-, tri-, tetra-, and hepta-/octa-repeats were 55.2% (132), 24.6% (59), 5.4% (13), and 0.8% (2), respectively (Table 2). We identified 132 di-nt repeat sequences, which consisted of $(AT)_n$, $(AC)_n$, and $(AG)_n$. Of these, the $(AG)_n$ and $(AC)_n$ repeats showed distinct predominance at 30.5% (73/239) and 21.7% (52/239), respectively. In comparison, $(AT)_n$ (2.9%, 7/239) had a relatively low frequency that is typical of other di-nt repeats. This relative predominance of $(AG)_n$ repeats is consistent with the distribution of microsatellites reported previously for barley, maize, rice, sorghum, and wheat (Temnykh et al., 1999; Kantety et al., 2002). Tri-nt motifs that repeated four or more times included $(AAT)_n$, $(AAC)_n$, $(AAG)_n$, $(ACC)_n$, and $(AGG)_n$. Of these, $(ACC)_n$ repeats were most frequent (7.5%, 18/239), followed by $(AAG)_n$ (5.8%, 14/239) and $(AAC)_n$

**Table 2.** Classification of repeat structures for the microsatellite sequences.

| Repeat unit | Repeat sequence | Synonym[a] | Observed no. | Total no. |
|---|---|---|---|---|
| Di-nt | AT | TA | 7 | 132 |
| | AC | CA, TG, GT | 52 | |
| | AG | GA, TC, CT | 73 | |
| Tri-nt | AAT | ATT, TTA, ATA, TAA, TAT | 6 | 59 |
| | AAC | ACA, CAA, TTG, TGT, GTT | 11 | |
| | AAG | AGA, GAA, TTC, TCT, CTT | 14 | |
| | ACC | CCA, CAC, TGG, GGT, GTG | 18 | |
| | AGG | GGA, GAG, TCC, CCT, CTC | 6 | |
| | Others | ACT, CGA, GCA, TCA | 4 | |
| Tetra-nt | AAAT | AATA, ATAA, TAAA, ATTT, TTTA, TTAT | 3 | 13 |
| | AAAG | AAGA, AGAA, GAAA, TTTC, TTCT, CTTT | 2 | |
| | AGAT | TAGA, ATAG, GATA, ATCT, TCTA, TATC | 2 | |
| | ACAT | TACA, ATAC, CATA, ATGT, TGTA, GTAT | 2 | |
| | AATT | ATTA, TTAA, TAAT | 3 | |
| | Others | GCTT | 1 | |
| Hepta-nt | AGAAGAG | CTCTTCT | 1 | 1 |
| Octa-nt | GAAAAAAA | TTTTTTTC | 1 | 1 |
| Complex repeat | | - | 33 | 33 |
| Total | | | | 239 |

[a]Same repeat sequences with sequence in column to the left.

(4.6%, 11/239). Although $(ACG)_n$ served as a bait in our library construction, we observed only one case of an $(ACG)_n$ repeat motif. Tetra-nt repeat motifs were identified from 13 different clones (5.4%, 13/239), and included $(AAAT)_n$, $(AAAG)_n$, $(AGAT)_n$, $(ACAT)_n$, and $(AATT)_n$. Although we did not use $(AGG)_n$, $(AATT)_n$, or the hepta- to octa-nt repeats as baits when preparing the microsatellite-enriched library, we identified six $(AGG)_n$, three $(AATT)_n$, and two different hepta- and octa-nt microsatellite-containing sequences. The rates of perfect repeats, compound motifs, and interrupted repeats were determined to be 72.8% (174/239), 6.6% (16/239), and 20.5% (49/239), respectively.

## DISCUSSION

Although *A. senticosus* is one of the most important medicinal herbs, supporting a vast commercial market in East Asia, the available genetic resources for its categorization and characterization remain largely insufficient. This species has not been actively analyzed as a genetic model system, so complete genomics information must still be determined. A number of microsatellites are thought to be necessary for the purpose of positional cloning of genes of interest and the physical mapping of quantitative trait loci (QTLs) for useful genetic traits. Screening after the preparation of a microsatellite-enriched genomic library is an effective method for identifying novel microsatellites (Brondani et al., 1998); however, such markers in *A. senticosus* are essentially unavailable. Therefore, our objective was to construct a library in order to fabricate a number of microsatellites.

Independent colonies from this first-ever documented, organized microsatellite-enriched library were estimated at approximately 5 X $10^5$ cfu, a level somewhat low compared with commercially available general genomic libraries. This might have resulted because only a few genomic fragments containing microsatellite sequences were collected. We did obtain a high rate of microsatellite-containing motifs (33.9%), an insert detection ratio of 91.7%, and an observed insert-size range of 0.2 to 1.2 kb. Likewise, our previously reported success in constructing a microsatellite-enriched genomic library of *Panax ginseng* relied on an identical strategy (Kim et al., 2007). Here, we detailed our isolation of 251 (33%) new microsatellite-containing sequences from insert detected clones (91.7%), a result that supports our claim that the microsatellite-enriched library was indeed appropriately prepared.

To identify the microsatellite motifs, we directly sequenced plasmids from randomly chosen colonies without performing colony hybridization. Our library screen revealed that 33.9% (403/1190) of the clones had microsatellite-containing sequences, a rate comparable to recovery efficiencies obtained by Brondani et al. (1998) from a microsatellite-enriched library by using plaque hybridization, in which the enrichment step yielded 10 to 40% of the clones containing a microsatellite. Although adding another hybridization step when selecting positive colonies (plaques) might have enhanced our ratio of clones with microsatellite motifs by up to 70%, the alternative method we have outlined in this report is sensitive to time and to cost.

We identified 239 different microsatellite-containing sequences, with di-nt repeat units being the most abundant,

**Table 3.** Repeat structures of the identified microsatellite-containing sequences.

| Repeat sequences | Microsatellite sequences (GenBank accession no.) |
|---|---|
| AT | AS463(EF594129), AS502(EF594141), AS682(EF594193), AS947(EF594240), AS1158(EF594271), AS1158-1(EF594270), AS1232(EF594292) |
| AC | AS17(EF594064), AS23(EF594066), AS34(EF594070), AS146(EF594084), AS146-1(EF594085), AS157(EF594086), AS189(EF594088), AS256(EF594095), AS292(EF594101), AS304(EF594105), AS318(EF594106), AS400(EF594117), AS417(EF594121), AS471(EF594133), AS486(EF594136), AS502-1(EF594142), AS514(EF594144), AS561(EF594158), AS567(EF594161), AS573(EF594164), AS578(EF594166), AS588(EF594169), AS621(EF594179), AS686(EF594196), AS710(EF594201), AS716(EF594202), AS718(EF594204), AS722(EF594205), AS726(EF594206), AS727(EF594207), AS728(EF594208), AS737(EF594211), AS743(EF594214), AS747(EF594216), AS803(EF594227), AS807(EF594228), AS808(EF594229), AS829(EF594236), AS936(EF594239), AS952(EF594241), AS1110(EF594256), AS1119(EF594261), AS1121(EF594262), AS1122(EF594263), AS1131(EF594265), AS1144(EF594267), AS1174(EF594273), AS1200(EF594278), AS1207(EF594280), AS1236(EF594294), AS1270(EF594297), AS1303(EF594300) |
| AG | AS49(EF594075), AS90(EF594079), AS130(EF594082), AS141(EF594083), AS195(EF594089), AS251(EF594092), AS255(EF594094), AS273(EF594097), AS284(EF594098), AS291(EF594100), AS295(EF594103), AS367(EF594111), AS394(EF594113), AS398(EF594115), AS408(EF594119), AS431(EF594124), AS463-1(EF594130), AS466(EF594131), AS467(EF594132), AS477(EF594134), AS482(EF594135), AS496(EF594138), AS497(EF594139), AS498(EF594140), AS505(EF594143), AS528(EF594146), AS531(EF594147),AS532(EF594148), AS543(EF594150), AS545(EF594151), AS548(EF594152), AS553(EF594156), AS566(EF594159), AS571(EF594162), AS576(EF594165), AS584(EF594168), AS589(EF594170), AS596(EF594172), AS602(EF594173), AS604(EF594174), AS630(EF594180), AS635(EF594182), AS649(EF594185), AS680(EF594190), AS691-1(EF594198), AS695(EF594199), AS735(EF594210), AS742(EF594212), AS753(EF594218), AS790(EF594226), AS816(EF594232), AS828(EF594234), AS828-1(EF594235), AS830(EF594237), AS1069(EF594246), AS1071(EF594247), AS1080(EF594248), AS1096(EF594252), AS1106(EF594255), AS1134(EF594266), AS1155(EF594268), AS1158-2(EF594269), AS1166(EF594272), AS1179(EF594275), AS1204(EF594279), AS1208(EF594281), AS1211(EF594283), AS1214(EF594284), AS1222(EF594287), AS1226(EF594290), AS1231(EF594291), AS1294(EF594298), AS1295(EF594299) |
| AAT | AS86(EF594077), AS361(EF594109), AS571-1(EF594163), AS659(EF594186), AS765(EF594223), AS1188(EF594277) |
| AAC | AS98(EF594080), AS254(EF594093), AS257(EF594096), AS293(EF594102), AS327(EF594107), AS361-1(EF594110), AS420(EF594122), AS521(EF594145), AS755(EF594222), AS1103(EF594254), AS1214-1(EF594285) |
| AAG | AS88(EF594078), AS301(EF594104), AS460(EF594128), AS548-1(EF594153), AS620(EF594178), AS671(EF594188), AS675(EF594189), AS691(EF594197), AS774(EF594225), AS990(EF594243), AS1087(EF594249), AS1088(EF594250), AS1092(EF594251), AS1115(EF594259) |
| ACC | AS1(EF594061), AS23-1(EF594067), AS27(EF594068), AS46(EF594074), AS62(EF594076), AS121(EF594081), AS229(EF594091), AS286(EF594099), AS399(EF594116), AS410(EF594120), AS552(EF594155), AS612(EF594175), AS660(EF594187), AS685-1(EF594195), AS809(EF594230), AS1113(EF594258), AS1208-1(EF594282), AS1232-1(EF594293) |
| AGG | AS42(EF594072), AS339(EF594108), AS685(EF594194), AS695-1(EF594200), AS1181(EF594276), AS1224(EF594288) |
| Other tri-nt | AS30(EF594069), AS566-1(EF594160), AS765(EF594223), AS1177(EF594274) |
| AAAT | AS22(EF594065), AS440(EF594127), AS809-1(EF594231) |
| AAAG | AS394-1(EF594114), AS1043(EF594245) |
| AGAT | AS432(EF594125), AS681(EF594191) |
| ACAT | AS45(EF594073), AS188(EF594087) |
| AATT | AS631(EF594181), AS681-1(EF594192), AS1034(EF594244) |
| Other tetra-nt | AS6(EF594062) |
| AGAAGAG | AS643(EF594184) |
| GAAAAAAA | AS1102(EF594253) |
| [a]Complex repeats | AS13(EF594063)-$(TG)_7(TA)_6$, AS35(EF594071)-$(TA)_6(TATG)_5$, AS220(EF594090)-$(TC)_4$-$N_9$-$(TC)_6$, AS375(EF594112)-$(TC)_{15}(AC)_9$, AS403(EF594118)-$(TG)_8$-$N_4$-$(TG)_3$-$N_2$-$(TG)_5$, AS438(EF594126)-$(TG)_6$-$N_2$-$(TG)_6$, AS491(EF594137)-$(TG)_6$-$N_{10}$-$(TG)_3$, AS542(EF594149)-$(TG)_5$-$N_2$-$(TG)_{10}$, AS551(EF594154)-$(TG)_4$-$N_{12}$-$(TG)_5$, AS557(EF594157)-$(TG)_4$-$N_6$-$(TG)_3$, AS582(EF594167)-$(TG)_4$-$N_2$-$(TG)_{10}$, AS590(EF594171)-$(TA)_8$-$N_8$-$(TA)_4$, AS613(EF594176)-$(TA)_5(TG)_9$, AS613-1(EF594177)-$(TC)_{12}$-$N_7$-$(TG)_7$, AS640(EF594183)-$(TC)_6$-N-$(TC)_3$, AS717(EF594203)-$(TG)_4$-$N_4$-$(TG)_{10}$, AS730(EF594209)-$(AG)_7$-$N_{18}$-$(TCA)_4$, AS742-1(EF594213)-$(TC)_4$-$N_2$-$(TC)_5$, AS743-1(EF594215)-$(TC)_4$-$N_2$-$(TC)_4$, AS752(EF594217)-$(CT)_{22}(CA)_{10}$, AS753-1(EF594219)-$(TA)_4(CA)_{16}$, AS754(EF594220)-$(TA)_5(TG)_9$, AS754-1(EF594221)-$(TC)_{12}$-N-$(TC)_3(TG)_7$, AS773(EF594224)-$(TC)_{11}(TA)_8(CA)_4$, AS824(EF594233)-$(TTA)_{10}(TTG)_{13}(TTA)_6$, AS976(EF594242)-$(TG)_5$-$N_2$-$(TG)_5$, AS1111(EF594257)-$(TG)_{11}$-$N_4$-$(TG)_3$-$N_2$-$(TG)_5$, AS1116(EF594260)-$(GGT)_5$-$N_3$-$(TGT)_5$, AS1123(EF594264)-$(TG)_{12}$-$N_6$-$(TG)_5$, AS1216(EF594286)-$(GA)_5$-$N_{15}$-$(GA)_5$, AS1225(EF594289)-$(TC)_4$-N-$(TC)_6$, AS1236-1(EF594295)-$(TA)_5(TG)_8$, AS1242-(EF59496)-$(TG)_5(CG)_4(TG)_9$ |

[a]Complex repeat motifs were represented with each repeated structure.

at 55.2% (132/239). This ranking of frequencies was followed by tri-nt repeat units at 24.6%, tetra-nt repeats at 5.4%, and other repeats at 0.8% (Table 2). For the specific repeat structures themselves, the $(AG)_n$ motif was most common (30.5%), then $(AC)_n$ (21.7%).

This predominance of $(AG)_n$ repeats in the *A. senticosus* genome is consistent with the microsatellite distributions already reported for barley, maize, rice, sorghum, and wheat (Temnykh et al., 1999; Kantety et al., 2002). However, the distribution ratios found in our study do not reflect the actual distributions of microsatellites throughout the genome examined here because we were limited to only 12 kinds of oligomers, with a 2- to 4-nt repeat unit as bait for microsatellite enrichment (Table 1). Although the $(AT)_n$ repeat motif is known as the most frequent di-nt repeat in plants (Cardle et al., 2000), it is not usually used as a probe for microsatellite-enrichment procedures due to its self-complementary nature (Cai et al., 2003). Here, by using that particular motif as a probe for our library construction, we obtained a relatively lower number of $(AT)_n$ repeat motifs (2.9%, 7/239) than either $(AG)_n$ or $(AC)_n$, again because of self-complementation.

Even though some microsatellite motifs, such as $(AGG)_n$, $(AATT)_n$, $(AGAAGAG)_n$, and $(GAAAAAAA)_n$ repeats are not used for constructing library, we did calculate an unexpectedly significant ratio (4.6%, 11/239) for these motifs, perhaps resulting from non-specific clones. Generally, conducting a plasmid hybridization step with SSR probes prior to sequence analysis enhances the ratio of microsatellite-containing sequences. However, we omitted that step and used direct sequencing after T-vector sub-cloning.

In conclusion, we have now created a microsatellite-enriched genomic library for *A. senticosus*. A total of 239 new microsatellite-containing sequences were identified and classified according to their structure of repeat units (Tables 2 and 3). This information should be helpful in further studies that develop the genetic resources from that species.

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## LITERATURE CITED

Awang DV (1996) Siberian ginseng toxicity may be case of mistaken identity. Can Med Assoc J 155: 1237

Balasubramaniyan V, Kalaivani Sailaja J, Nalini N (2003) Role of leptin on alcohol-induced oxidative stress in Swiss mice. Pharmacol Res 47: 211-216

Bao Y, Zhou HF, Hong DY, Ge S (2006) Genetic diversity and evolutionary relationships of *Oryza* species with the B- and C-genomes as revealed by SSR markers. J Plant Biol 49: 339-347

Bonierbale MW, Plasisted RL, Tanksley SD (1988) RFLP maps based on a common set of clones reveal modes of chromosomal evolution in potato and tomato. Genetics 120: 1095-1103

Brondani RPV, Brondani C, Tarchini R, Grattapaglia D (1998) Development, characterization and mapping of microsatellite markers in *Eucalyptus grandis* and *E. urophylla*. Theor Appl Genet 97: 816-827

Cai H-W, Yuyama N, Tamaki H, Yoshizawa A (2003) Isolation and characterization of simple sequence repeat markers in the hexaploid forage grass timothy (*Phleum pratense* L.). Theor Appl Genet 107: 1337-1349

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. Genetics 156: 847-854

Chambers GK, MacAvoy ES (2000) Microsatellites: Consensus and controversy. Comp Biochem Physiol B 126: 455-476

Cho U-H, Cho H-M, Kim H (1996) Detection of genetic variation and gene introgression in potato dihaploids using randomly amplified polymorphic DNA (RAPD) markers. J Plant Biol 39: 185-188

Fischer D, Bachmann K (1998) Microsatellite enrichment in organisms with large genomes (*Allium cepa* L.). Biotechniques 24: 796-802

Ha WY, Shaw PC, Liu J, Yau FC, Wang J (2002) Authentication of *Panax ginseng* and *Panax quinquefolius* using amplified fragment length polymorphism (AFLP) and directed amplification of minisatellite region DNA (DAMD). J Agric Food Chem 50: 1871-1875

Hamilton MB, Pincus EL, Di Fiore A, Fleischer RC (1999) Universal linker and ligation procedures for construction of genomic DNA libraries enriched for microsatellites. Biotechniques 27: 500-507

Kantety RV, La Rota M, 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

Kim J, Jo BH, Lee KL, Yoon E-S, Ryu KH, Chung KW (2007) Identification of new microsatellite markers in *Panax ginseng*. Mol Cells 24: 60-68

Kurata N, Umehara Y, Tanoue H, Sasaki T (1997) Physical mapping of the rice genome with YAC clones. Plant Mol Biol 35: 101-113

Martin GB, Brommonschenkel SH, Chunwongse J, Frary A, Ganal MW, Spivey R, Wu T, Earle ED, Tanksley SD (1993) Map-based cloning of a protein kinase gene conferring disease resistance in tomato. Science 262: 1432-1436

Metzgar D, Bytof J, Wills C (2000) Selection against frameshift mutations limits microsatellite expansion in coding DNA. Genome Res 10: 72-80

Mihalov JJ, Marderosian AD, Pierce JC (2000) DNA identification of commercial ginseng samples. J Agric Food Chem 48: 3744-3752

Roder MS, Plaschke J, Konig SU, Borner A, Sorrells ME, Tanksley SD, Ganal MW (1995) Abundance, variability and chromosomal location of microsatellites in wheat. Mol Gen Genet 246: 327-333

Tanksley SD, Grandillo S, Fulton TM, Zamir D, Eshed Y, Petiard V, Lopes J, Beck-Bunn T (1996) Advanced backcross QTL analysis in a cross between an elite processing line of tomato and its wild relative *L. pimpinellifolium*. Theor Appl Genet 92: 213-224

Tautz D (1989) Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acids Res 17: 6463-6471

Temnykh S, Park WD, Ayers N, Cartinhour S, Hauck N, Lipovich L, Cho YG, Ishii T, McCouch SR (1999) Mapping and genome organization of microsatellites in rice (*Oryza sativa*). Theor Appl Genet 100: 697-712

Um JY, Chung HS, Kim MS, Na HJ, Kwon HJ, Kim JJ, Lee KM, Lee SJ, Lim JP, Do KR, Hwang WJ, Lyu YS, An NH, Kim HM (2001) Molecular authentication of *Panax ginseng* species by RAPD analysis and PCR-RFLP. Biol Pharm Bull 24: 872-875