Development of 34 New Microsatellite Markers from Actinidia arguta: Intra- and Interspecies Genetic Analysis

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ABSTRACT  The present study investigated the isolation and characterization of 34 polymorphic microsatellite markers developed from Actinidia arguta (Sieb. and Zucc.) Planch. ex Miq. var. arguta. These markers produced 349 alleles in eight Actinidia species, with an average of 10.3 alleles per locus. Observed heterozygosity ranged from 0.50 to 0.87 (mean = 0.72), and polymorphism information content ranged from 0.37 to 0.88 (mean = 0.69). The phylogenetic relationship obtained using microsatellite markers showed minor clustering and population differences among species while 38 A. arguta accessions fell into two subgroups. These newly developed polymorphic microsatellite markers will be very useful in sustainable genetic conservation, marker-assisted breeding, and classification of the Actinidia genus.

Keywords  Microsatellites, Actinidia arguta, Genetic diversity

INTRODUCTION

The genus Actinidia comprises 76 species and about 120 taxa of perennial, dioecious, viny, and deciduous fruit trees. Actinidia species have a wide range of distribution in eastern Asia that includes tropical equatorial and temperate regions (Ferguson and Huang 2007). Four of the 76 species have been domesticated as fruit crops: A. deliciosa, A. chinensis, A. arguta, and A. eriantha (Cui et al. 2002; Ferguson 1990). A. deliciosa (A. Chev.) C. F. Liang and A. R. Ferguson var. deliciosa is a green-fleshed kiwifruit, and A. chinensis Planch. var. chinensis is yellow-fleshed. Kiwifruit production has increased remarkably since the introduction of A. deliciosa seeds to New Zealand in the early 1900s, reaching more than one million tons in 2000 (Huang and Ferguson 2001).

Four Actinidia species, A. arguta, A. kolomikta, A. polygama, and A. rufa, are found in Korea (Cui et al. 2002; Ferguson 1990; Kim et al. 2003). A. arguta (Sieb. and Zucc.) Planch. ex Miq. var. arguta grows in the wild in mountainous and hilly terrain throughout Korea; it is tolerant to freezing and is resistant to various harmful insects. Because the exterior of the fruit is soft, smooth, and leathery, it can be eaten whole without peeling (Williams et al. 2003). Given its richness in various minerals and vitamin C, this fruit is commonly used in soft drinks and jams. It is also used as an anodyne, diuretic, antifebrile, and thirst-quencher in home remedies (Lee et al. 2004). Despite these beneficial traits and uses, commercial cultivars of A. arguta have not been available, and most plants are growing in the wild without much improvement. Although several attempts have been made to introduce the genes from A. arguta into A. deliciosa (Kim et al. 2008; Lee et al. 2004), the hybrids have not yet been widely accepted.
The *A. arguta* accessions have not been subjected to genetic diversity analysis. For the conservation and sustainable use of wild accessions, it is important to understand the genetic variation, genetic structure, and mating systems of natural populations. Given their high levels of polymorphism with codominance and their excellent reproducibility, microsatellite or simple-sequence repeat (SSR) markers have been preferred for use in the analysis of molecular diversity in conservation biology and molecular ecology (Park et al. 2009). Although microsatellite markers have been developed in the *Actinidia* species, this development has been restricted to the commercial species *A. chinensis* and *A. deliciosa* and has not included *A. arguta* (Huang et al. 1998; Korkovelos et al. 2003). Huang et al (1998) demonstrated that 40 microsatellite markers derived from the genomic library of *A. chinensis* were successful in the genomic DNA of *A. arguta*. Another study (Fraser et al. 2000) found that four of nine microsatellite markers derived from the genomic library of *A. deliciosa* were successful in the polymerase chain reaction (PCR) amplification of *A. arguta* accessions. The same group of researchers subsequently tested cross-species amplification among 21 *Actinidia* species with 20 expressed sequence tag (EST)-derived microsatellite markers (Fraser et al. 2005). Three of these markers failed in amplification, and three others were inconsistent in the amplification of *A. arguta* (4x) accessions.

In an effort to preserve the genetic diversity of *A. arguta* in Korea, we collected wild accessions throughout the country and stored the seeds in a short-term preservation facility. The seeds were screened for genetic redundancy before they were placed in a long-term storage facility. Because microsatellite markers are preferred for genetic diversity analysis, we developed microsatellite markers from the *A. arguta* genomic library. This report describes the new set of markers for genetic diversity analysis in *Actinidia* species and possible markers for sex determination in *A. arguta*.

MATERIALS AND METHODS

Plant materials and genomic DNA extraction

A total of 91 accessions from eight *Actinidia* species were used in this study (Table 1). The young leaves were acquired from Namhae Sub-Station of National Institute of Horticultural & Herbal Science which conserved diverse accessions of *Actinidia* species in experimental field. Total genomic DNA was extracted from ground power of young leaves by liquid nitrogen using the Plant DNAzol® Reagent (Invitrogen, Carlsbad, CA, USA), following the supplier’s protocols. The extracted DNA was quantified using an ultraviolet-visible (UV-Vis) spectrophotometer (ND-1000; NanoDrop, Wilmington, DE, USA).

Construction of an SSR motif-enriched library

A modified biotin-streptavidin capture method was used to construct an SSR motif-enriched library of *A. arguta* (Sieb. and Zucc) Planch. ex Miq. var. *arguta* genomic DNA (Kwon et al. 2005). Genomic DNA samples (10 µg) were digested with seven blunt-end-producing restriction enzymes (*EcoRV*, *DraI*, *SmaI*, *PvuI*, *AluI*, *HaeIII*, *RsaI*). The fully digested DNA samples were pooled and size-fractionated on 1.4% agarose gels. DNA fragments of 300 bp to 1,500 bp were eluted from the gels and were purified using a gel extraction kit (Qiagen, Hilden, Germany). Approximately 1 µg of DNA fragments was ligated with 1 µg of the double-stranded adaptor (AP11/AP12). The adaptor was prepared by mixing equal molar amounts of oligonucleotides AP11 (5’CTC TTG CTT AGA TCT GGA CTA-3’) and AP12 (5’-TAG TCC AGA TCT AAG CAA GAG CAC A-3’), heated to 94°C, then cooled to 25°C over a period of 4–5 h. Preamplification of adaptor-ligated DNA fragments was performed for 15 cycles of PCR in a 50-µl reaction volume with a single primer (AP11) and an annealing temperature of 56°C. The preamplified products were hybridized with a mixture of long (40–45 nucleotides) biotin-labeled repeat probes. Hybridization was performed for 2 h at 65°C in a reaction mixture (50 µl) that included 6× standard sodium citrate (SSC), 0.1% sodium dodecyl sulfate (SDS), ~100 ng of preamplified product, and 300 ng of each biotin-labeled oligo: (GA)$_{20}$, (CA)$_{20}$, (AG)$_{15}$, (GG)$_{15}$, (AAG)$_{15}$, (AAC)$_{15}$, and (AGG)$_{15}$. The hybridized DNA fragments were captured with 400 µg of streptavidin-coated magnetic beads (Promega, Madison, WI, USA) by incubating the mixture at 65°C with gentle agitation for 30 min. The beads were separated from the liquid using a
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Table 1. List of *Actinidia* species used in this study.

| Taxon | Number of accessions | Variety | Geographic location |
|-------|----------------------|---------|---------------------|
| *A. arguta* (Sieb. and Zucc.) Planch. ex Miq. var. arguta | 40 | | Korea |
| 63. K5-1-22 | 73. K5-3-6 | 83. K5-4-12 | 93. K5-10-1 |
| 64. K5-2-3 | 74. K5-3-7 | 84. K5-5-1 | 94. K5-10-2 |
| 65. K5-2-7 | 75. K5-3-8 | 85. K5-5-8 | 95. K5-10-3 |
| 66. K5-2-8 | 76. K5-3-12 | 86. K5-5-12 | 96. K5-10-5 |
| 67. K5-2-10 | 77. K5-3-13 | 87. K5-5-14 | 97. K5-10-8 |
| 68. K5-2-13 | 78. K5-3-18 | 88. K5-6-10 | 98. K5-11-1 |
| 69. K5-2-16 | 79. K5-4-3 | 89. K5-6-12 | 99. K5-11-2 |
| 70. K5-2-18 | 80. K5-4-7 | 90. K5-9-1 | 100. K5-11-3 |
| 71. K5-2-22 | 81. K5-4-8 | 91. K5-9-4 | 101. K5-11-8 |
| 72. K5-3-3 | 82. K5-4-10 | 92. K5-9-8 | 102. K5-12-6 |
| *A. chinensis* Planch. var. chinensis | 13 | | Korea |
| 14. SKK3 | 20. SKK10 | 104. Red-Kiwi | 106. Jesi-Gold |
| 16. SKK5 | 21. SKK11, 23. SKK13, 62. Jeju-Gold | 105. Hort16A | 115. Guem-Goi |
| 17. SKK7 (Gracies) | | 108. Yeo-San-Hyang | |
| 19. SKK9 (Chin Mei) | | | |
| *A. chinensis* Planch. var. chinensis | 6 | | China |
| 12. SKK1 | 30. S13 | 18. SKK8 (Tomuri) | 117. SKK12-2 |
| 13. SKK2 | 15. SKK4 | | |
| *A. deliciosa* (A. Chev.) C.F. Liang and A.R. Ferguson var. deliciosa | 25 | | New Zealand, Japan, China |
| 3. Hayward | 9. Golden Yellow (Wha-Pyung 2 Ho) | 116. Jin-Mi | |
| 4. Monty | 10. Sensation Apple (Jinkui) | 42. SKK43 (Seo-Hyang) | |
| 5. Matua | 37. SKK18 (Chieflain) | 43. SKK44 | 110. M51-2 |
| 6. Tomuri | 39. SKK23 | 46. SKK 61 | |
| 34. Sun-WuKong | 41. SKK34 (Mi-Lyang 2 Ho) | 109. M51-1 | |
| *A. eriantha* Benth. | 3 | 50. S10 | 103. Bi-Dan | China, Korea |
| *A. arguta* var. purpurea (Rehd.) C.F. Liang | 1 | 24. S3 | | China |
| *A. kolomikta* (Maxim. and Rupr.) Maxim. | 1 | 25. S4 | | China |
| *A. macrosperma* C.F. Liang | 1 | 27. S7 | | China |
| *A. rufa* (Sieb. and Zucc.) Planch. ex Miq. | 1 | 61. Seom-Darae | | Korea |

Magnetic stand (Promega) and were washed five times in 300 µl 6× SSC/0.1% SDS (1× SSC = 150 mM NaCl, 15 mM sodium citrate, pH 7.0) at room temperature with gentle agitation. After stringent washing, all samples were briefly washed in 5× SSC to remove the SDS, and DNA fragments were eluted with 50 µl of dH2O at 90°C for 5 min. Final elutions (5 µl) were amplified for 15 cycles of PCR using the AP11 primer. After checking the gel, the amplified DNA products were cloned into pGEM-T Easy Vector (Promega) and were transformed into *Escherichia coli* cells through electroporation. Recombinant colonies were identified by blue or white colony selection on
lysogeny broth (LB) plates containing ampicillin, 5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside (X-gal), and isopropyl β-D-1-thiogalactopyranoside (IPTG).

**DNA sequencing and SSR primer design**

A total of 762 white colonies were randomly selected from the enriched library. Plasmid DNA was purified with the QIAprep Spin Miniprep Kit (Qiagen), and nucleotide sequencing was performed using an ABI 3100 DNA sequencer with a BigDye terminator kit (Applied Biosystems, Foster City, CA, USA). SSR motif elucidation and primer design were performed using the ARGOS program (Kim 2004).

**PCR amplification**

The “M13 tail at its 5′ end” PCR method was used to measure the size of the PCR products (Schuelke 2000). PCR amplification was performed in a total volume of 20 µl containing 2 µl of genomic DNA (10 ng/µl), 0.2 µl of the specific primer (10 pmol/µl), 0.4 µl of M13 universal primer (10 pmol/µl), 0.6 µl of normal reverse primer, 2.0 µl of 10× PCR buffer (Takara, Tokyo, Japan), 1.6 µl of deoxynucleoside triphosphate (dNTP; 2.5 mM), and 0.2 µl of Taq polymerase (5 unit/µl; Takara). The PCR amplification conditions were as follows: 94°C for 3 min, followed by 30–33 cycles at 94°C (30 s), 50–55°C (45 s), 72°C (45 s), then 15 cycles at 94°C (30 s), 53°C (45 s), and 72°C (45 s), and final extension at 72°C for 20 min. PCR was performed in PTC-220 thermocyclers (MJ Research, Waltham, MA, USA). The PCR products of three microsatellites were mixed together in a ratio of 6-FAM:HEX:NED (fluorescent dyes) = 1:3:4, which varied depending on the amplification intensity for individual markers as determined on an ABI PRISM 3130xl genetic analyzer (Applied Biosystems). A phylogenetic dendrogram was constructed using the unweighted pair-group method with arithmetic averages (UPGMA) with PowerMarker (Liu and Muse 2005) and MEGA4 (Tamura et al. 2007) software.

**RESULTS**

**Development of SSR markers from A. arguta**

A total of 762 clones were randomly picked from the primary transformation plates and were sequenced (Table 2). After excluding redundant clones (n = 35) and truncated clones with SSR motifs near their ends (n = 162), 565 clones were analyzed for primer designing. Of these, 140 clones were suitable for designing primer pairs for PCR amplification of the SSR motifs. Clones containing <12 nucleotides in the SSR motifs were not included in the
primer design. Dinucleotide repeat-motif-containing clones were predominant (86.1%) among the 565 clones, with 864 dinucleotides, 87 trinucleotides (8.7%), and 53 tetranucleotides or more (5.8%) clones (Table 3).

### Variation among Actinidia accessions
SSR variations were sought using the 140 selected primer pairs with a core collection of 91 Actinidia accessions representing diverse regions and species (Table 1). This collection is maintained at the National Institute of Horticultural & Herbal Science (RDA, Korea). Intra- and interspecies accession polymorphisms were present in 34 primer pairs (Supplementary Table 1). The number of repeating units among these polymorphic SSR loci varied from 6 to 23, and the length of the repeat region varied from 12 bp in GB-AA-017 [(CT)₆] to 174 bp in GB-AA-065 [(GA)₁₁(GAA)₂₃] (Table 4). Whereas 26 (82.6%) polymorphic loci contained di-nucleotide repeating motifs, only three

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**Table 2.** Efficiency of the procedure adopted for the identification of microsatellite markers in *A. arguta*.

| Procedural step                  | Number (percentage) |
|----------------------------------|---------------------|
| Sequenced clones                 | 762                 |
| Redundant clones                 | 35 (4.6%)           |
| Unique clones                    | 727 (95.4%)         |
| SSR clones                       | 565 (74.1%)         |
| Truncated clones (5’ or 3’ end)  | 162 (21.3%)         |
| Fewer than 12 nucleotides        | 263 (34.5%)         |
| Primer pairs designed            | 140 (18.4%)         |
| Polymorphic loci                 | 34 (24.3%)          |

²) percentage based on the designed primer pairs.

**Table 3.** Frequency and type of di-, tri- and ≥ tetra-nucleotide repeats isolated from the *A. arguta* microsatellite-enriched library.

| Repeat unit | Repeat class | Number of microsatellite loci | Polymorphic loci |
|-------------|--------------|-------------------------------|------------------|
|             |              | Number | Percentage | Number | Percentage |
| Di-nucleotide | AC/CA/TG/GT  | 238     | 23.7%     | 8      | 17.4%      |
|              | AG/GA/TC/CT  | 603     | 60.1%     | 30     | 65.2%      |
|              | AT/TA        | 22      | 2.2%      | 1      | 2.2%       |
|              | CG/GC        | 1       | 0.1%      |        |            |
| Total        |              | 864     | 86.1%     |        |            |
| Tri-nucleotide | AAC/ACA/CAA | 15      | 1.5%      | 1      | 2.2%       |
|              | AAG/AGA/GAA  | 44      | 4.4%      |        |            |
|              | AAT/ATA/TAA  | 4       | 0.4%      | 2      | 4.3%       |
|              | ACC/CCA/CAC  | 3       | 0.3%      |        |            |
|              | AGC/CGA/GAC  | 4       | 0.4%      |        |            |
|              | AGG/GGA/GAG  | 14      | 1.4%      |        |            |
|              | ATC/TCA/CAT  | 1       | 0.1%      |        |            |
|              | CCG/CGC/GCC  | 2       | 0.2%      |        |            |
| Total        |              | 87      | 8.7%      |        |            |
| ≥ Tetra-nucleotide | 53     | 5.8%      | 5      | 10.9%    |
| Total repeat motifs | 1004 | | 46 | |
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Table 4. Characteristics of 34 polymorphic microsatellite loci for *Actinidia* species.

| Marker   | GeneBank accession | Repeat motif      | Primer sequence          | Size (bp) | Ta (°C) | A   | MAF  | He   | Ho  | PIC |
|----------|-------------------|-------------------|--------------------------|-----------|---------|-----|------|------|-----|-----|
| GB-AA-005 FJ647762 | (CT)$_{17}$        | F: AGTTGTGCATCCAAAGGCA R: CAGTGGGGTGAGAGAACGA | 192-218   | 57      | 12    | 0.32 | 0.73 | 0.78 | 0.76 |
| GB-AA-012 FJ647763 | (TG)$_{13}$        | F: TCACCAACACTATTTCCGCG R: ATCCGCTTTCCTTAGTGC | 154-176   | 58      | 10    | 0.49 | 0.47 | 0.71 | 0.68 |
| GB-AA-015 FJ647764 | (AG)$_{19}$        | F: CCTTGTCTGATGGGAAT R: ATGGCATTTGTTGCG | 267-293   | 58      | 14    | 0.24 | 0.89 | 0.83 | 0.83 |
| GB-AA-017 FJ647765 | (CT)$_{6}$         | F: AAAGTGTGACGACGTGACAA R: TGAGAGAGAGAGCTGC | 170-182   | 58      | 2     | 0.54 | 0.00 | 0.50 | 0.37 |
| GB-AA-018 FJ647766 | (CA)$_{8}$, (GGA)$_{4}$ | F: ACCATGACACAGTGGAA R: TCCAGTGCCTTAAGCCCC | 147-171   | 58      | 11    | 0.23 | 0.75 | 0.81 | 0.80 |
| GB-AA-024 FJ647767 | (GA)$_{19}$        | F: AGGGACCCACAGAGGACAA R: ATTCGCTTCCTGGACACAC | 155-181   | 58      | 12    | 0.40 | 0.49 | 0.75 | 0.74 |
| GB-AA-054 FJ647768 | (GA)$_{15}$        | F: ACCAAAAACACCTGCTCT R: TGACCCGATTTGGGACATC | 186-214   | 58      | 15    | 0.23 | 0.87 | 0.85 | 0.84 |
| GB-AA-065 FJ647769 | (GA)$_{11}$, (GAA)$_{23}$ | F: ATGGAACCCCATTTGAG R: CCCAGGAGCAACATTTAGG | 212-238   | 58      | 11    | 0.43 | 0.60 | 0.74 | 0.72 |
| GB-AA-069 FJ647770 | (GGGA)$_{6}$, (GA)$_{8}$ | F: CGTTCTCCCTGACCAATTT R: CGTTCACCACCATCACCA | 196-258   | 58      | 11    | 0.43 | 0.67 | 0.74 | 0.71 |
| GB-AA-080 FJ647771 | (GA)$_{15}$        | F: CCAATCAAAGATGGCACAG R: TGGGAGTGGAAACTGGA | 179-213   | 58      | 11    | 0.26 | 1.00 | 0.82 | 0.80 |
| GB-AA-084 FJ647772 | (GA)$_{15}$        | F: CATTGCAACACGACAAAT R: AGTCGAGCTCAGGGAGAG | 233-259   | 58      | 12    | 0.22 | 0.96 | 0.83 | 0.82 |
| GB-AA-088 FJ647773 | (CT)$_{7}$         | F: TCTGTTTGTTCCTTCCACCA R: GTGGTACGGCACTTCC | 177-197   | 57      | 8     | 0.45 | 0.56 | 0.69 | 0.63 |
| GB-AA-091 FJ647774 | (CT)$_{19}$        | F: TGACCTAAGGGGCAGGACAA R: GGAATCTAATCCTCAGGACA | 201-223   | 58      | 11    | 0.38 | 0.62 | 0.74 | 0.69 |
| GB-AA-094 FJ647775 | (AC)$_{15}$        | F: ACAGGGGGAACATCGTGTC R: GTGGGATATAACGCGGGA | 208-220   | 57      | 6     | 0.38 | 0.41 | 0.68 | 0.63 |
| GB-AA-096 FJ647776 | (GA)$_{8}$, (GA)$_{8}$ | F: TTTGGGTACAAAGACGGCC R: AAAATTCTTTCCACCGGCC | 229-235   | 58      | 3     | 0.52 | 0.01 | 0.50 | 0.39 |
| GB-AA-302 FJ647777 | (AG)$_{13}$        | F: TCATTGATAAATGGCAGG R: GGGCCTTGGACACGACAG | 269-311   | 58      | 11    | 0.43 | 0.31 | 0.74 | 0.72 |
| GB-AA-303 FJ647778 | (CT)$_{15}$        | F: TGGGGCATTATGTGCTAT R: TCAAACCTTTGGCCTTC | 156-258   | 58      | 12    | 0.33 | 0.35 | 0.75 | 0.73 |
| GB-AA-304 FJ647779 | (AG)$_{11}$        | F: CAGTCACAAATTERGGTCAG R: GTGGAAACCATGCCCC | 202-364   | 58      | 13    | 0.41 | 0.15 | 0.67 | 0.64 |
| GB-AA-308 FJ647780 | (CCCT)$_{4}$, (GGGT)$_{2}$ | F: TATGGTGCAGCAAGGAGGA R: ATTGGCAACGTTCTGGGC | 265-304   | 58      | 8     | 0.32 | 0.36 | 0.77 | 0.75 |
| GB-AA-331 FJ647781 | (GA)$_{15}$, (CAGA)$_{4}$ | F: CCATCTTTTGTTGCCTTTG R: TTGGTCAATCATGGCC | 259-303   | 57      | 13    | 0.28 | 0.59 | 0.83 | 0.80 |
| GB-AA-333 FJ647782 | (TG)$_{12}$        | F: AAGTTCAATCTAGCGCAG R: ATTCGATTGACGGCCTA | 242-276   | 58      | 6     | 0.49 | 1.00 | 0.67 | 0.61 |
| GB-AA-337 FJ647783 | (GA)$_{10}$, (AC)$_{6}$ | F: TATCTCTGCGGTTCCTCTC R: AGCTCAACCAAGAAGG | 290-332   | 58      | 10    | 0.36 | 0.53 | 0.74 | 0.72 |
| GB-AA-340 FJ647784 | (CCT)$_{5}$        | F: AAAGGAATTCGCCCTCTCAA R: GGCTGACAAGAAGCG | 287-296   | 58      | 4     | 0.65 | 0.68 | 0.49 | 0.45 |
Intra- and interspecies relationships obtained from SSR profiles

Proportions of shared alleles were used to calculate genetic distances between all pairwise combinations among the 91 Actinidia accessions used in this study. A phylogenetic dendrogram based on the SSR profiles clearly shows two major groups at a genetic distance of 0.45 (Fig. 1). The first group (GI) comprised A. chinensis, A. deliciosa, A. eriantha, A. kolomikta, A. macrosperma, and A. rufa. The accessions of the four latter species (A. eriantha, A. kolomikta, A. macrosperma, and A. rufa) did not form a subspecific clade, but were intermixed in each GI clade. The GII clade included A. arguta was clearly distinguished from first group (GI) and formed two major subclades at a genetic distance of 0.25.
DISCUSSION

In this study, 762 DNA sequences of *A. arguta* were retrieved from a microsatellite-enriched library, but only 140 (18.4%) were used to design the SSR primers. The majority of DNA sequences (81.6%) were not suitable for primer design; 55.8% had SSRs at the 5′ or 3′ end and fewer than 12 nucleotides in the SSR motifs; and 4.6% were duplicate or redundant DNA sequences (Table 2). This result was consistent with those reported for *Sorghum bicolor* and cassava (*Manihot esculenta* Crantz), wherein 70% and 45% of the clones, respectively, had SSRs too close to the cloning sites at the 5′ or 3′ end (Mba et al. 2001; Taramino et al. 1997). Similarly, redundant DNA sequences consisting of the same SSR locus or showing more than 95% similarity in flanking sequences were found in 20% of cassava accessions (Mba et al. 2001), 16% of perennial ryegrass (*Lolium perenne* L.) accessions (Jones et al. 2001) and 2.2% in avocado (*Persea americana* Mill.) accessions (Ashworth et al. 2004). Most redundancies were due to cloning, locus duplication, or allelism and were from the same microsatellite enrichment library. Ashkenazi et al (2001) also reported that some conserved potato (*Solanum phureja* Juz. & Buk.) DNA sequences flanking microsatellite regions were too short to permit the design of an appropriate primer. Earlier studies reported that the AT/TA repeat was the most frequent type of SSR in plants, followed by the AG/GA/TC/CT repeat (Danin-Poleg et al. 2001; Wang et al. 1994; Yu et al. 1999). In this study, the most frequent type of microsatellite repeat was AG/GA/TC/CT (60.1%), followed by AC/CA/TG/GT (23.7%), AAG/AGA/GAA (4.4%), and AT/TA (2.2%; Table 3). However, the frequency of a microsatellite repeat may vary with species and genus. For instance, Ashworth et al (2004) reported that AG and

![Phylogenetic dendrogram of the varieties and species in *Actinidia* based on SSR amplification profiles.](image)
ATG were the most frequent repeats in avocado.

The SSR genetic diversity observed among Actinidia accessions in our study can be compared to the diversities among A. chinensis and A. deliciosa that have been measured using other SSR sets (Korkovelos et al. 2008). The average numbers of alleles and PIC in our analysis were 10.3 and 0.69, respectively. Similarly, Korkovelos et al. (2008) reported averages of 7.8 ± 3.2 alleles and PIC = 0.739 ± 0.158. These values are higher than those detected in cucumber (Cucumis; average 2.4 alleles/locus, PIC = 0.28) and common buckwheat (Fagopyrum; average 5.9 alleles/locus, PIC = 0.48) (Danin-Poleg et al. 2001; Ma et al. 2009). Thus, the SSR markers among 91 Actinidia accessions in the present study were effective for assessing genetic diversity and for understanding population structure.

The species relationship derived from our cluster analysis is consistent with other reports (Huang et al. 2002; Li et al. 2002). In particular, the clustering results confirmed the previously established close relationship between A. deliciosa and A. chinensis (Cipriani et al. 1998; Huang et al. 1997; Testolin and Ferguson 1997), revealing A. chinensis as a progenitor of A. deliciosa (Cipriani et al. 1998). Others have considered A. arguta to be promising for the development of cultivars (Ferguson 1999; Nishiyama 2007). These major species were unambiguously classified into two groups in the present study. However, A. kolomikta and A. macrosperma were included in Group I in our analysis. This result contrasts with a previous analysis using RAPD markers that placed these species with A. arguta (Huang et al. 2002). This result also contrasts with a study using matK gene and internal transcribed spacer (ITS) sequences that found a lesser genetic distance between these species and A. arguta than with other groups (Li et al. 2002). The morphological features of A. kolomikta and A. macrosperma are also more similar to those of species in the A. arguta group (Ferguson 1990) than to those of other groups. The grouping result may be due to the limited amplification of transferability in SSR markers. Although most Actinidia species were successfully amplified with all 34 SSR markers, the amplifications of transferability in A. kolomikta and A. macrosperma were 80% and 57%, respectively. The transferability of SSRs to other Actinida species showed average value of 77% ranged from 57% (A. macrosperma) to 90% (A. chinensis). A little higher transferability than other genus such as Prunus (mean=59%) and Allium (mean=59%) reveals that genetic distances are relatively low between tested Actinidia species (Lee et al. 2011; Wang et al. 2012).

These newly developed polymorphic microsatellite markers will be very useful for selection in marker-assisted breeding, genetic conservation, and classification of the Actinidia genus. The sex-related markers identified in our study are also of major importance to kiwifruit breeders and the produce industry because they allow the distinction of female plants at the seedling stage and have great potential utilization in marker-assisted selection in this species.

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