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# Cross-amplification of SSR markers developed from *Allium sativum* to other *Allium* species

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ABSTRACT

For genetic analysis of the genus *Allium*, which is composed of diverse species, we acquired 50 transferable and polymorphic microsatellite markers from *A. sativum* and tested them for transferability in five *Allium* species. Among the 50 simple sequence repeat (SSR) loci, the dinucleotide motif was the most prevalent, with a ratio of 50% (25/50), and (GT)n was more frequent than (GA)*n* within the dinucleotide motif. The average number of amplified alleles ranged from 1.452 to 1.910 and the accessions of *A. tuberosum* had a maximum of 4.8 alleles per accession with the *GB-AS-104* SSR marker. Whereas *A. porrum* belonging to the *Allium* section revealed 73.0% transferability, *A. altaicum* and *A. fistulosum* appertaining to different sections showed low transferability, with a ratio of 47.6% and 48.0%, respectively. The phylogenetic results for these SSR markers did not deviate from previous classifications of the genus *Allium*. As the rate of successful amplification of SSR markers generally correlates with genetic distance, these SSR markers are potentially useful in the analysis of genetic relationships between or within *Allium* species.


## 1. Introduction

The genus *Allium* includes diverse crops containing approximately 700 species, such as garlic (*A. sativum*), onion (*A. cepa*), leek (*A. porrum*), chive (*A. schoenoprasum*), and shallot (*A. ascalonicum*) (Stearn, 1992; Berg et al., 1996). *Allium* species have a unique taste and odor and have been cultivated for consumption and medicinal use for more than 4000 years (Milner, 1996). The medicinal functions of *Allium* species can be attributed to diverse bioactive natural compounds such as organosulfur compounds, which have anti-platelet and potential cancer-preventing activities (Goldman et al., 1996; Bianchini and Vainio, 2001). Although *Allium* is one of the largest plant genera, the genetic relationships between *Allium* species have not been clearly identified because of the diverse ecotypes within various taxonomic and geographical groups (Hanelt et al., 1992; Stearn, 1992). According to previous classification studies, the genus *Allium* is grouped into six subgenera and 46 sections. Most of the commonly cultivated types belong to the *Allium* (garlic, leek) and *Rhizirideum* (bulb onion, bunching onion, Chinese chive) subgenera (Hanelt et al., 1992; Ohri et al., 1998).

Recently, various molecular marker systems have been used to resolve taxon identity within the genus *Allium*, such as RAPD, RFLP, and nuclear sequences of ribosomal ITS regions (Wilkie et al., 1993; Friesen et al., 1999; Mes et al., 1999). The results of these molecular analyses support previous classification and taxonomic groupings; for example, RAPD and RFLP revealed that *A. fistulosum* originated from *A. altaicum* (Wilkie et al., 1993; Friesen et al., 1999; Nguyen et al., 2008). Microsatellites (simple sequence repeats, or SSRs) distinguished by the size difference of alleles may be generated by the mis-replication of repeated sequences (Richards and Sutherland, 1994). SSR markers are highly reproducible, genetically co-dominant, and multi-allelic, and they reveal a significant level of allelic diversity by PCR (Ishii et al., 2001). Many crops lack sufficient sequence information, and studies of SSR transferability to related taxa have used these markers for minor crops and wild relatives (Varshney et al., 2005). In one study of the six genera of the Leguminosae family, PCR products were generated in 286 reactions and represented 60% (286/475) transferability (Mace et al., 2008).

SSR markers have been reported in specific *Allium* species, such as garlic (*A. sativum*), bunching onion (*Allium fistulosum* L.) and bulb onion (*A. cepa*), and have been used for genetic analysis within

*Abbreviations:* SSR, simple sequence repeat or microsatellite.

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**Table 1**
List of *Allium* accessions used in this study.

| Subgenera | Section | Species | Crop names | Serial no_accession no (Origin) |
|---|---|---|---|---|
| *Rhizirideum* | *Cepa* | *A. altaicum* | Wild *Allium* | 1_IT204110 (Mongolia), 2_IT204135 (Mongolia), 3_IT119820 (Russia), 4_IT215057 (Russia), 5_IT215058 (Russia) |
| | | *A. fistulosum* | Welsh onion | 6_IT100547 (Korea), 7_IT100548 (Korea), 8_IT100550 (Korea), 9_IT160631 (Korea), 10_IT175840 (Korea) |
| | *Butomissa* | *A. tuberosum* | Chinese chives | 11_IT204027 (Korea), 12_IT204031 (Korea), 13_IT204036 (Korea), 14_IT204041 (Korea), 15_IT204047 (Korea) |
| *Allium* | *Allium* | *A. porrum* | Leek | 16_IT216901 (Korea), 17_IT216903 (Korea) |
| | | *A. sativum* | Garlic | 18_Danyang-9 (Korea), 19_Danyang-611 (Russia), 20_Danyang-628 (Korea) |

species (Fischer and Bachmann, 2000; Wako et al., 2002; Song et al., 2004; Ma et al., 2009b). However, the number of applicable SSR markers within the genus *Allium* and studies of transferability have been insufficient to analyze the diversity of *Allium* species. Thus, we isolated SSRs from *Allium sativum* and cross-amplified them in several *Allium* species to analyze genetic relationships and transferability between *Allium* species. From among 129 microsatellite primer pairs, we selected 50 transferable primer pairs (including eight polymorphic SSRs in garlic) and applied them to five *Allium* species: two *Cepa* sections (*A. altaicum* and *fistulosum*), one *Butomissa* section (*A. tuberosum*), and two *Allium* sections (*A. porrum* and *sativum*) (Hanelt et al., 1992; Ohri et al., 1998).

## 2. Materials and methods

### 2.1. Plant materials and DNA extraction

A total of 20 accessions (five *Allium* species), provided by the Rural Development Administration (Korea), were used (Table 1). Species and subgroup identification information was in accordance with the available descriptors (Hanelt et al., 1992; Ohri et al., 1998). Genomic DNA was extracted from leaves using the modified CTAB method (Dellaporta et al., 1983) and quantified with a UV–Vis spectrophotometer (ND-1000; NanoDrop, Wilmington, DE, USA).

### 2.2. Construction of an SSR-enriched library and design of SSR primers

In a previous study, we constructed the SSR-enriched library from the garlic genome using the modified biotin–streptavidin capture method (Ma et al., 2009b). Briefly, genomic DNA of *A. sativum* var. *sativum* was digested using six restriction enzymes; *Alu*I, *Dra*I, *Hae*III, *Rsa*I *Eco*RV, and *Nru*I. The digested DNA was size-fractionated on 1.4% agarose gels and eluted from gels ranging from 300 to 1500 bp. After adaptor ligation and PCR amplification, the DNA fragments were hybridized with a mixture of biotin-labeled SSR probes: $(GA)_{20}$, $(CA)_{20}$, $(AT)_{20}$, $(GC)_{20}$, $(AGC)_{15}$, $(GGC)_{15}$, $(AAG)_{15}$, $(AAC)_{15}$, and $(AGG)_{15}$. The hybridized DNA fragments were captured using streptavidin-coated magnetic beads (Promega, Madison, WI, USA) and were cloned into the pGEM-T Easy vector (Promega). Recombinant colonies on an LB plate were chipped and sequenced using an ABI3100 DNA sequencer. SSR MANAGER (Kim, 2004) was used to identify the SSR motif and design the primer pairs covering these regions.

### 2.3. PCR amplification

The M13-tail PCR method, which attaches M13 at the 5′-end region of forward primers, was used to measure the size of amplified products for economical genotyping (Schuelke, 2000). PCR amplification was conducted in a total volume of 20 μl containing 2 μl genomic DNA (20 ng/μl), 0.2 μl of the specific primer (10 pmol/μl), 0.4 μl M13 universal primer (10 pmol/μl), 0.6 μl normal reverse primer, 2.0 μl 10× h-Taq PCR buffer (Solgent, Korea), 1.6 μl dNTP mix (2.5 mM), and 0.3 μl h-Taq polymerase (2.5 unit/μl; Solgent). PCR reaction conditions were as follows: 94 °C (3 min); then 30 cycles each of 94 °C (30 s), 56 °C (45 s), and 72 °C (1 min); followed by 10 cycles of 94 °C (30 s), 53 °C (45 s), and 72 °C (1 min); and a final extension step at 72 °C for 10 min. PCR was performed in PTC-200 thermocyclers (MJ Research, Waltham, MA, USA), and the SSR alleles were resolved using an ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems) with an internal size standard, Genescan-500 ROX (6-carbon-X-rhodamine) molecular size standards (35–500 bp). Assignment of appropriate alleles in amplified products was done at the base-pair level using GeneScan and Genotyper software ver. 3.7 (Applied Biosystems).

### 2.4. Data analysis

In the cluster analysis, each detectable amplified allele with primers was treated as a character unit and coded into a binary format: 1 or 0 for presence or absence, respectively. A dendrogram was constructed using the NTSYSpc program (Rohlf, 2000) and the unweighted pair-group method with arithmetic averaging. Winboot (Yap and Nelson, 1996) was used for the bootstrapping analysis. In the principal coordinate analysis, Two-dimensional grouping pattern of *Allium* accessions was constructed with two principal coordinates using GenAlEx ver. 6.1 (Peakall and Smouse, 2006).

## 3. Results

### 3.1. Development of SSR markers and the number of alleles in the A. sativum

A total of 564 SSR clones (redundancy = 13.12%) were sequenced. We designed 128 primer pairs comprising SSR motifs, as the remainders were not suitable because of biased or invalid SSR motives. In addition to 8 polymorphic SSR loci in garlic accessions, 42 SSR loci applicable to the study of genetic relationships in the genus *Allium* were selected, for a total of 50 SSR loci (Ma et al., 2009b). Core repeating motifs were categorized as dinucleotide,

**Table 2**
Type of core repeating motif.

| Type | Core repeating motif | Number of clones (%) |
|---|---|---|
| Di-nucleotide | GA/TC | 3 (6) |
| | GT/AC | 12 (24) |
| | TA | 10 (20) |
| | Total | 25 (50) |
| Tri-nucleotide | CCG/CGG | 7 (14) |
| | TTC/GAA | 3 (6) |
| | CCT/AGG | 2 (4) |
| | GGA/TCC | 3 (6) |
| | TCA/TGA | 2 (4) |
| | TTG/CAA | 2 (4) |
| | TTA/TAA | 1 (2) |
| | GGT/ACC | 2 (4) |
| | Total | 22 (44) |
| Tetra-nucleotide | TTTA/TAAA | 3 (6) |
| Total | | 50 (100) |